**Eukaryotic Gene Expression: Basics & Benefits**
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**Lecture No. # 17**
**Regulation of gene expression by second messengers other than cAMP**

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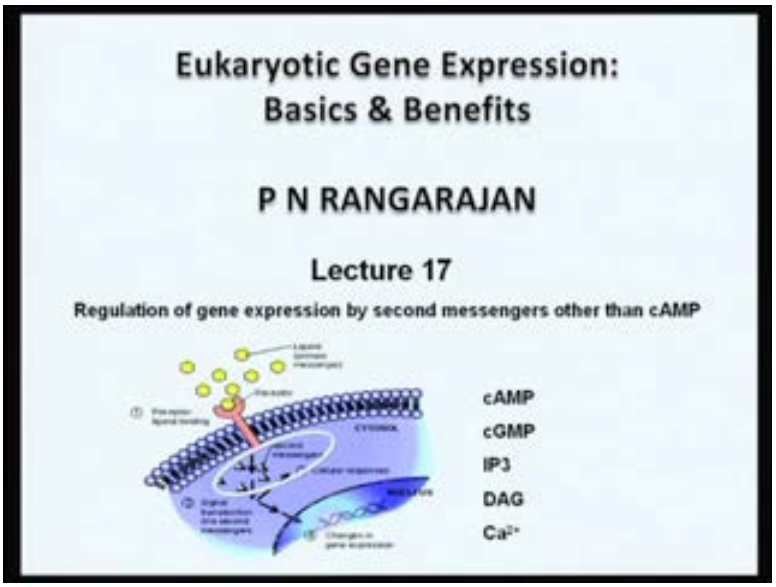


We will continue our lecture series on the regulation of eukaryotic gene expression. In the last 2 lectures, we have been trying to understand how molecules which either interact with membrane receptors or which enter into the cell interact with soluble receptors. Ultimately, transducer signals to the nucleus leading to alternation of gene expiration as I.. To begin with, we have been looking at the membrane receptor signal. In the last 2 lectures, we have actually covered how molecules which are water soluble. Therefore, cannot penetrate the lipid barrier interact with specific membrane receptors and then, how these molecules transitive signals leading to activation of gene expression.

To begin with the various kinds of membrane receptors who have been focusing our attention on one particular group of receptors will be the gene protein couple receptors which are 7 transmembrane receptor, serpentine receptors to which specific small molecules like epinephrine or even peptide molecules like insulin binds. It results in the activation of specific G proteins which are trimeric G proteins and these trimeric G proteins then get activated and as a result, the alpha subunit disassociates. In one case, it

goes and activates adenylate cyclase leading to activation of cyclic AMP synthesis of cyclic AMP. Cyclic AMP then goes and activates protein kinase and protein kinase A now goes inside the nuclear and phosphorylates transcription factors like CREB leading to activation of cyclic AMP responsive genes. So, this is one pathway that we have studied in the last class.

Now, cyclic AMP is not the only second messenger which is synthesized in the cells. There are number of other second messengers has shown in this particular cartoon. We have cyclic GMP where we know thrall triphosphate, triphosphate diacylglycerol calcium. All these things are very important second messengers in transducing signals into the nucleus and activating specific gene expression programs. So, what we will do today is, to look at regulation of gene expression by second messengers other than cyclic AMP. So, let us take a look at and then, see how molecules like cyclic GMP inostal, triphosphate, diacylglycerol and calcium when they get activated or how they go ahead and influence gene expression programs first going to the crux of today's lecture.

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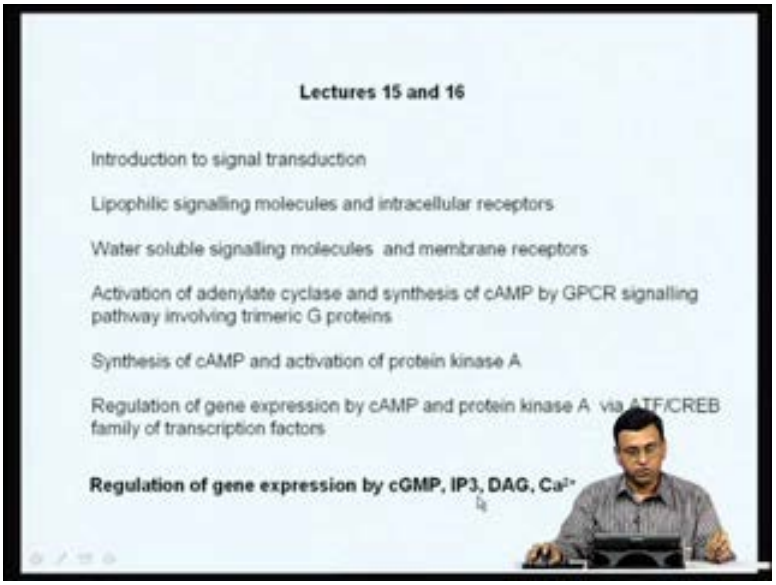


So, what I just summarized, what we have discussed in the last 2 classes when we began our lectures on signal transduction pathways. We started with a very brief introduction about signal transduction, we discussed briefly about lipophilic signal molecules, how they defuse through the cell membrane and interact with the certain intracellular receptors and how water soluble ligands which since they cannot penetrate the lipid

barrier. They interact with the membrane receptors and activate signal transaction pathways and there are wide varieties of membrane receptors with which these molecule interacts and we discussed about one particular class of membrane receptors, namely the GPCR's or G protein coupled receptors.

When these G protein couple receptors are activated, they in turn activate trimeric G proteins and the G alpha submitted now goes and activates adenylate cyclase leading to the synthesis of cyclic AMP. Cyclic AMP in turns goes and activates protein kinase A and then, cyclic AMP protein kinase A via the ATF family of transcription factors activates genes which are in response to cyclic AMP which contains cyclic AMP response elements in their promoters. This is what we have discussed in the last 2 classes. So, today let us see how regulation of gene expression is to be carried out by cyclic G n pinositol triphosphate diacylglycerol calcium.

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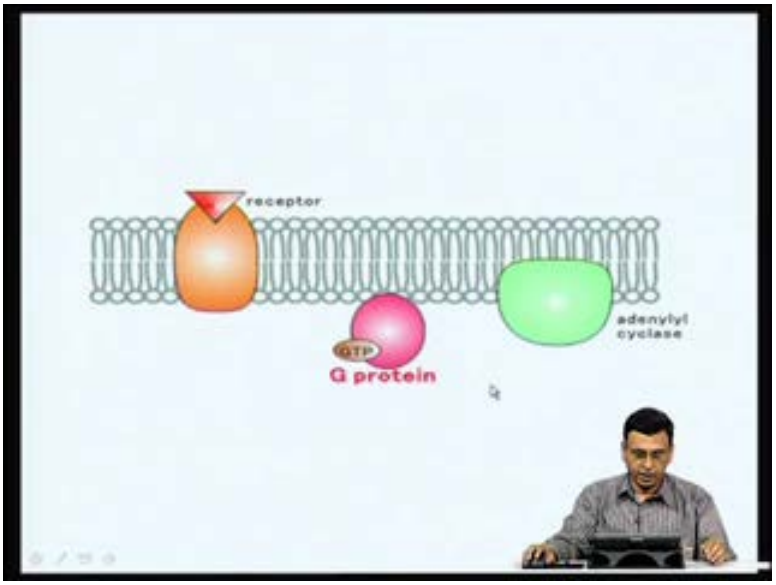


Now this is what we have discussed so far. When a hormone binds to a GPCR, it viscous the activation of a G protein which is trimeric G protein and the G protein now binds to Gtp and gets activated and the Gtp bound form it now goes and interacts to the adenylate cyclase. Adenylate cyclase is now activated and adenylate cyclase will now convert GMP into cyclic AMP and this cyclic AMP thus synthesis now activates protein kinase A and protein kinase A in turn activates phosphorylates molecules like CREB and then, resulting in activating of transcription.

So, this is the summary of the last class about how binding of your hormone molecule to a cell surface receptor results in the sense of cyclic AMP leading to the activation of protein kinase.

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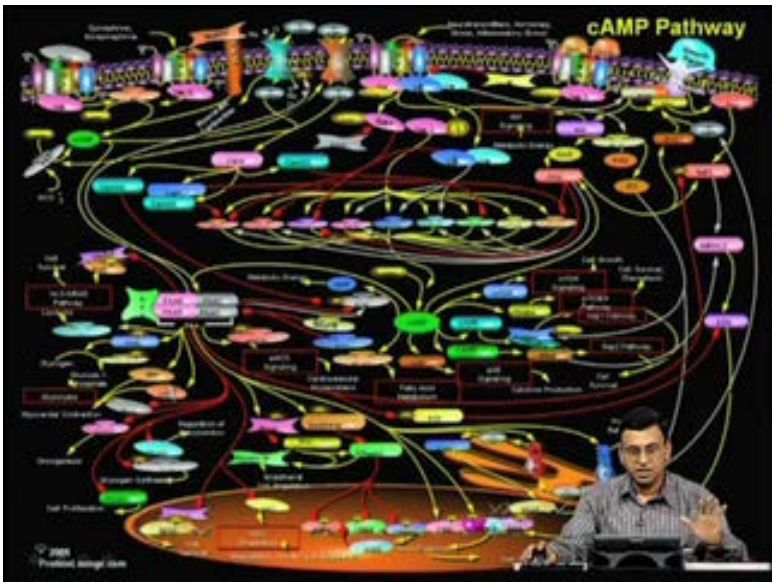


Now, I also discussed that we have discussed a very small part of cyclic AMP signaling because our focus is primarily on the regulation of gene expression. Cyclic AMP once synthesized does a number of other things like activation of metabolic enzymes and number of other pathways, but we focused our attention primarily on regulation of gene expiration.

So, we confined our self to only one part of this complex pathway. I have shown here namely how cyclic kp once activated, how it activates protein kinase A and then, how protein kinase A goes inside the nuclear and phosphorylates a number of transcription factors leading to activation of gene expression. We have not really discussed a number of other things that cyclic AMP does because we are not really interrupt, we want to focus primarily on the regulation of gene expression by second messengerss and therefore, confined our self to only one particular aspect of this signaling pathway.

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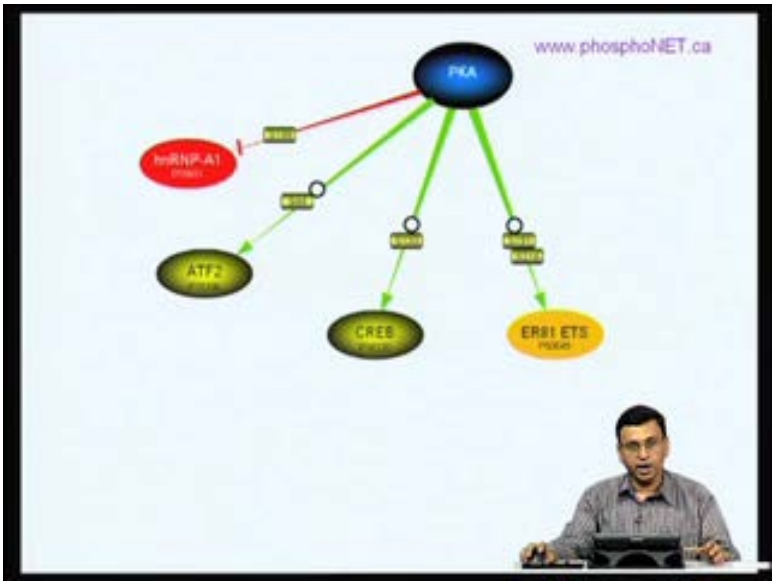


I also focused primarily my attention in the last class on how protein kinase A activates ATF CREB family of transcription factors and I have shown clearly that pk phosphorylates serine 133 of CREB and it also phosphorylates serine 44 of the ATF2. As a result, both of them now get activated and bind to cyclic AMP response element in the active transcription of gene. We also discussed about how some of the mutant, some of the variants of the CREB which arise because of alternate splicing molecule like icier which log the transactivation domain when they get activated in response to PKA from the p 2 promoter of CREB. They actually act as a negative regulators of genes now more and more such transcription factors which are being regulated by PKR being discovered for example in addition to the ATF CREB family of transcription factor.

A protein called ERAT1ETS is also have been shown to dephosphorylated by protein kinase A and therefore, this also plays a very important role in number of physiological processes and similarly, another transcription factor called h and rnpa1 which plays a very important RNA processing again. This is also a target for PKA. It gets phosphorylated on serine in 199. That also is activated by PK signal A. So, although we focus our attention primarily on ATF CREB family of transcription factors which are the targeted cyclic AMP and PKA, more and more transcription factors are being reorganized as target s or PKA and are being identified in being studied.

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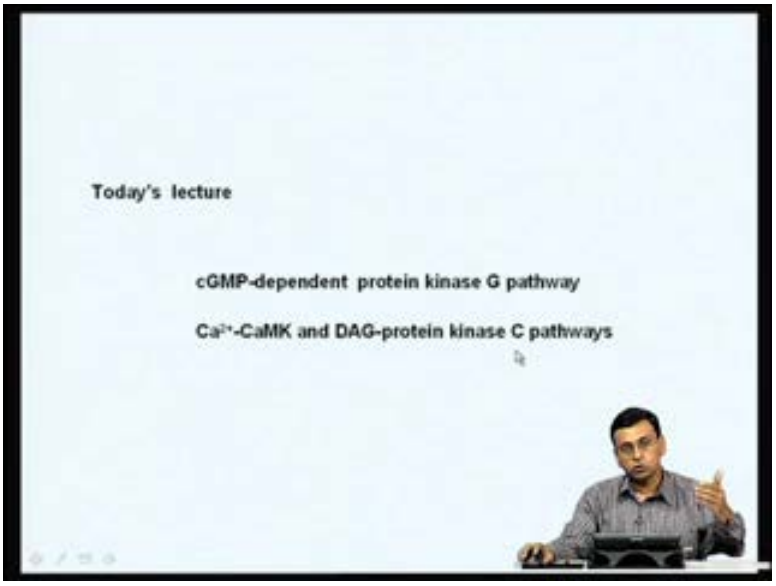


So, having studied this cyclic AMP dependent protein kinase A pathway, today's lecture we will focus primarily on cyclic GMP dependent protein kinase G pathway and how calcium and calmodulin kinase as well as diacylglycerol activates another protein kinase, very important protein kinase called protein kinase C and how this will lay the foundation for us to discus about how protein kinase C 1 act. Once it gets activated, how it goes and regulates gene expression programs.

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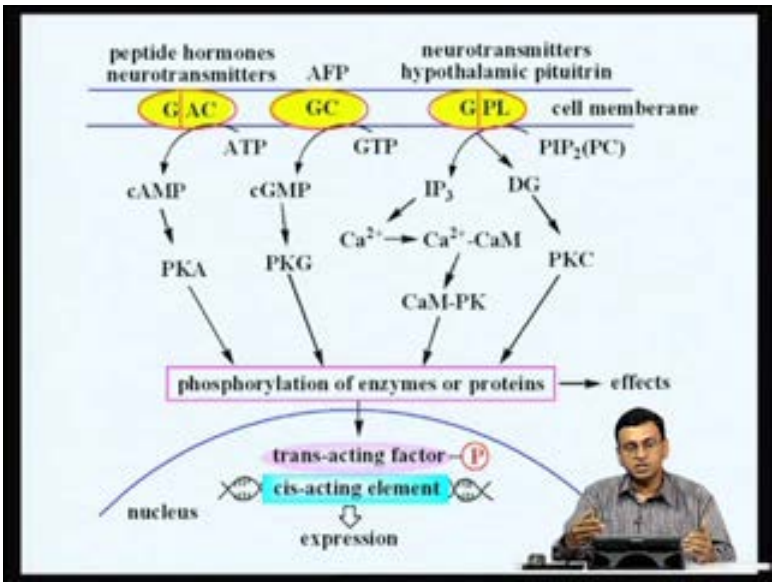

So, in this particulate cartoon, I have basically summarized what all we are going to discuss today and what we have discussed yesterday in the last class. For example, there are three major signaling pathway that are going to focus on in the last class and today's class what we have discussed so far is the activation of membrane respecters which activate trimaric gene protein in which the G alpha sub unit interacts primarily with the adenylate cyclase. As a result, GMP is converted into cyclic AMP which then activates protein kinase A that now goes inside and phosphorylates a number of transcription factor leading to activation or repression of gene expression. This is what we have discussed now.

What we are going to discuss today is about a receptor which actually posses intrinsic Guanylate activity. Now, in this case, the trimaric G protein interacted with the adenylate cyclase leading to the activation of synthesis of cyclic AMP, but the cyclic GMP synthesis actually involves a membrane receptor which has an intrinsic Guanylate cyclase activity. As a result, the binding of ligand to the receptor activates its Guanylate cyclase activity and as a result, this Guanylate cyclase now convert GTP to cyclic GNP which then activates protein kinase G and which then goes inside and then, phosphorylates transcription factor leading to activation of gene expression programs.

So, this is one pathway going to discuss now. The other thing we are going to discuss is molecules like neuron transmitters and certain hypothalamic hormones. They activate a different set of G proteins which just like this G protein here activate adenylate cyclase and here, you got activation of Guanylate cyclase. This particular G protein now activates phospholipase C and phospholipase C now converts phosphatidyl phosphatidyl bis inositol inositol inositol bisphosphate into inositol phosphate 3 and diacylglycerol.

These two are very important second messengerss. Inositol triphosphate now goes and releases calcium from the endoplasmic reticulum and calcium thus release now activates the calcium calmodulin kinase and this calcium and calmodulin kinase then goes and activates the cam kinases are calmodulin protein kinase which again goes and either phosphorylates a number of enzymes are proteins either outside nuclease or transcription factor inside the nuclear leading to activation of specific physiological process. The diacylglycerol also is a very important activated protein kinase C. Both calcium and diacylglycerol together can also protein kinase C and protein kinase C again can

phosphorylate a number of proteins including transcription factors leading to activation repression of target genes.

So, these are the genes, major signal transaction pathways that we are going to discuss today. As well as yesterday that in the last class, we have covered which involves trimic G proteins and how some of this trimeric G proteins activates adenylate cyclase and some of the G proteins activate phospholipase C in the passes second messengerss like cyclic AMP, cyclic GMP inositol triphosphate. All these second messengerss synthesize which amplify the signal go and then, phosphorylate a number of enzymes and proteins including transcription factors leading to alternation of gene expression.

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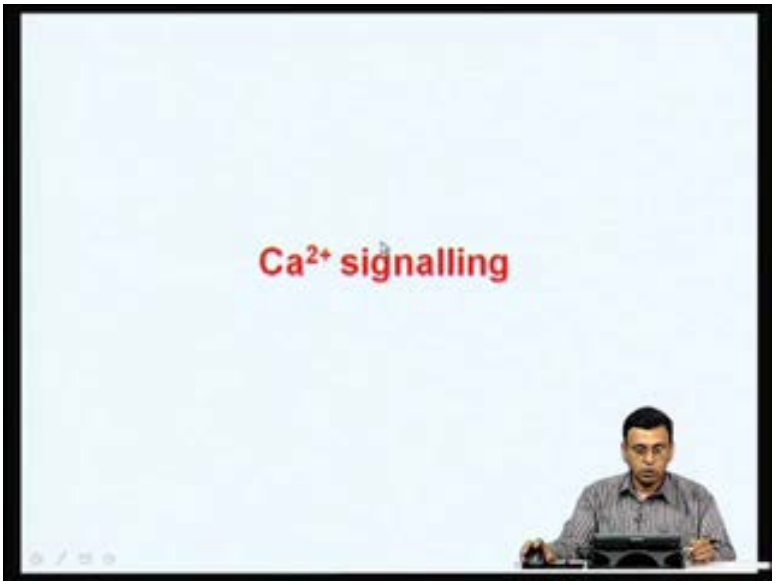


So, let us now see how does calcium regulates gene expression. What is the important feature of calcium signaling?

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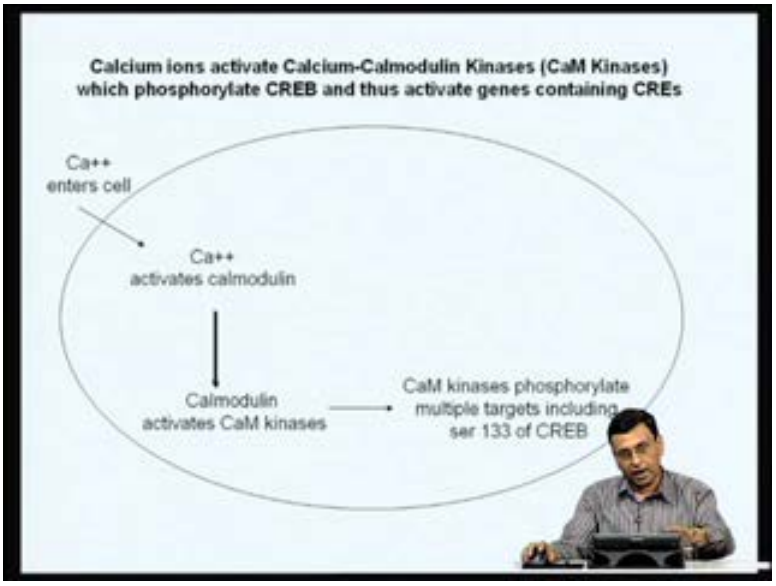


Now, we have already discussed in the previous class how calcium can also activate CREB. So, CREB can be activated not only by cyclic AMP, CREB can also be activated by multiple signaling pathways that is what we discussed in the last class and one of them is calcium. So, once the calcium enters the cell, calcium activates calmodulin or calcium can also be released from the endoplasmic reticulum. Once the intracellular calcium, cytosol calcium increases calcium now activates calmodulin. It is a very important protein and calmodlin inter activates cam kinases and cam kinases phosphorylate multiple targets including serine 133 of cyclic AMP response binding protein. In fact, we discussed in detail in the last class, how the cam kinases especially in nervous system plays very important role in the activation of CREB and how cyclic AMP as well as cam kinases activate CREB and in turn, it involves in the long term memory and so and so forth. So, let us now see in addition to activation of CREB, how calcium influence gene expression. That is what is going to be the focus of today's class.

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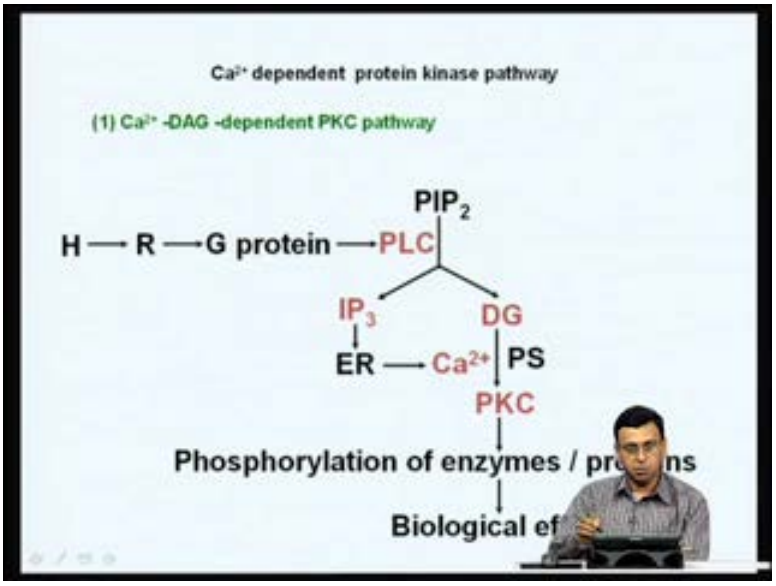


Now, there are two different mechanisms which calcium can activate gene expression programs. One is what is called the diacylglycerol dependentprotein kinase C pathway and another is using the cam kinases pathway which we discussed. So, let us now have the brief schematic that actually gives you the crux of what we are going to discuss now. Once the hormone binds to a specific cell surface receptor, it activates result in the activation of a G protein trimeric G protein and this G alpha sub unit of the G protein now activates phospholipase C.

So, in the case of the cyclic AMP pathway, the G proteins, the G alpha subunit activates the adenylate cyclic and as a result got synthesis of cyclic AMP, but here the G protein activates phospholipase C and as a result, you get the synthesis of two different second messengerss, namely inositol triphosphate and diacylglycerol. Now, phosphoto inositol bis phosphate is a very important lipid molecule that remains anchor to the cell membrane and phospholipase C now cleaves the phosphatidylinositol bisphosphateinto inositol triphosphate and diacylglycerol. Now, the inositol triphosphate thus synthesized now goes into the endoplasmic reticulum and then, mobilizes the calcium release causes the calcium release and now, the calcium release goes and activates the protein kinase C along with diacylglycerol and the protein kinase C thus activated now phosphorylates a number of enzymes proteins including transcription factor leading to specific phylogical have that.

So, calcium by virtue of activating leading to synthesis of diacylglycerol and calcium release and activation of protein kinase C can influence specific gene expression programs. So, the protein kinase C that is now synthesized can go and phosphorylate a number of enzymes are proteins including transcription factors resulting in specific biological effects.

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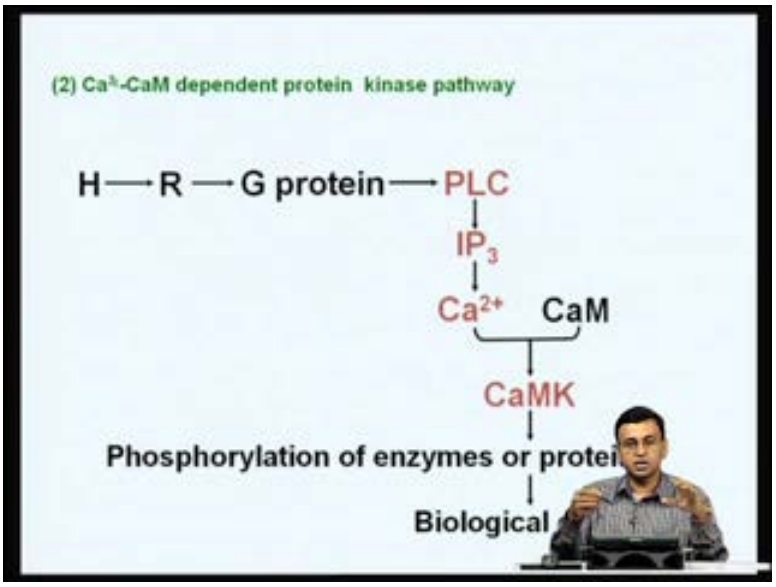


So, this is one pathway by which calcium can activate gene expression programs and as we already discussed the calcium when the same hormones are ligands binds to specific to subtractive gene proteins, the phospholipase C that is activated now results in the mobilization of formation of inositol triphosphate which mobilizes the calcium, store now the calcium that is now released in the last slide. We have mentioned how it goes and activates protein kinase C. The same calcium can also now combine with calmodlin and then, the calcium calmodlin now can bind to calcium on binding to calmodulin. In terms of calmodulin, the cam kinase gets activated and these camkinase now goes and number of enzymes and proteins leading to specific biological effects.

So, calcium can activate both protein kinase C on one hand along with diacylglycerol and calcium, along with calmodulin can also activate cam kinases. So, calcium activates signals access a signal transduction molecules in two access second messenger two different pathways. One is calcium together with diacylglycerol can activate protein kinase C which can phosphorylate wide varieties of sub states leading to activation high

performance using gene expression programs. The calcium can also combine with activate calmodulin and calmodulin now can activate cam kinases and cam kinases can activate different set of proteins and transcription factor leading to specific biological effects.

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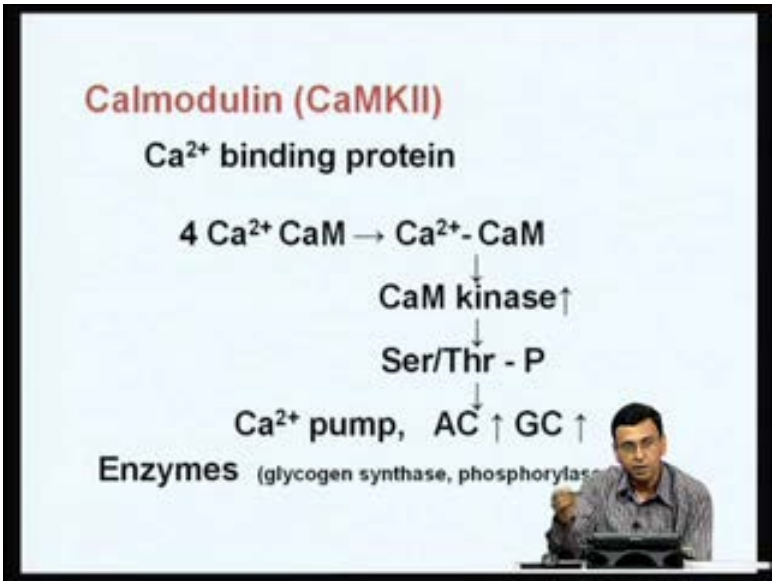


Now, what is calmodulin? Calmodulin is a calcium binding protein and four molecules and four calcium ions can actually buy into one molecule of calmodulin. The calcium calmodulin complex now goes and binds to calcium calmodulin kinase and induce a conformational change the calmodulin kinase and now, the calmodulin kinase now can go and phosphorylate specific certain in 3n residues of target proteins. As a result, one of the physiological effects that happens in the cam kinase C increase in the intrasolar calcium levels, especially from the endoplasmic reticulum. Calcium is related to cytoplasm. There is increase in calcium reserve inside the cytoplasm of plasma and it can also well in the activation of adenylate cyclases Guanylate cyclase so on and so forth. So, a number of physiological process like glycosine synthase, phosphorylates kinases etcetera are all activated by cam kinases and there are also number of transcription factors including CREB which are activated by cam kinases.

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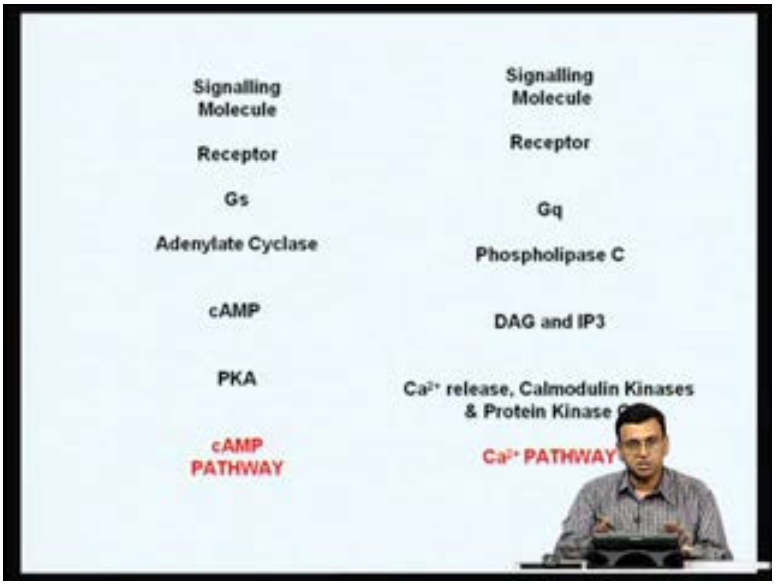


So, there are two pathways we have discussed so far, especially involving the G protein coupled receptors. In one pathway, when a signaling molecule binds to the GPCR specific receptor, it now activates in the activation of a trimeric G protein and in the alpha subunit, especially the G s alpha G alpha s subunit activates adenylate cyclase leading synthesis of cyclic AMP which does instance of protein kinase A and protein kinase A now goes and phosphorylate a number of transcription factors leading to activation by pressure of target genes.

So, this is what is called the cyclic AMP pathway and we also now discussed just the calcium pathway, where different kind of signaling molecules when the receptor with their combined receptors that is using the activation of a different class of G proteins and here, the G sub G alpha subunit is called the G q which now instead of activating adenylate cyclase here, it activates phospholipase C and phospholipase C now converts inositol diphosphate into diacylglycerol inositol diphosphate.

Diacylglycerol and inositol triphosphate now release and results in the calcium release and the calcium together. Diacylglycerol activate protein kinase C and calcium also binds to calmodulin results in the activation of calmodulin kinases and these two kinases together can go and phosphorylate number of proteins including transcription factor leading to activation or repression of target genes. So, these are the two major signal

transaction pathways we have discussed so far how cyclic AMP and calcium are acting as very important second messengers.

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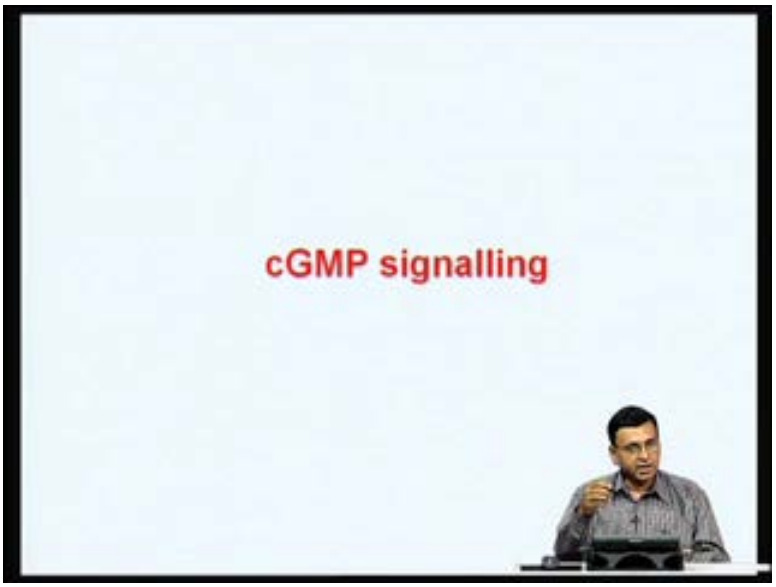


Now, let us spend some time to understand in addition to cyclic AMP. There is also very important cyclic nucleotide, namely cyclic GMP and how cyclic GMP acts as a signaling molecule.

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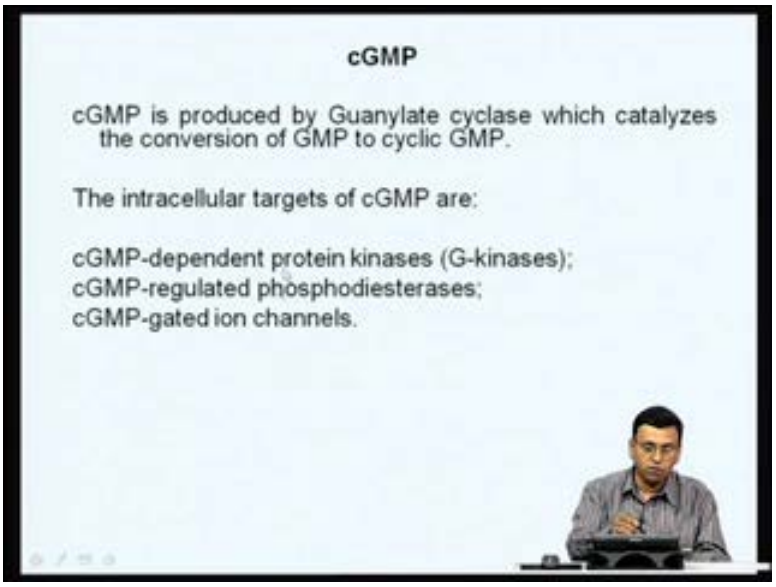


Now, cGMP as we have just discussed now is produced by Guanylate cyclase which catalyzes the conversion of GMP to cyclic GMP. Now, GMP the Guanylate cyclase are

actually in two forms. A membrane bound form and a soluble form. We will discuss in just a minute now what the intra-cellular targets of this cyclic GMP are. One cyclic GMP synthesis cellular trafficking inside the cells, they can go and activate cyclic GMP dependent protein kinases. These are usually referred to as G kinases and it can also activate cyclic GMP regulate phosphodiesterases which actually cleve cyclic GMP and cyclic GMP is also in opening up of specific ion channels, especially in the nervous system. So, cyclic GMP has a number of cellars functions including activation of specific proteins kinases phosphodiesterases as well as opening our closing of ion channels.

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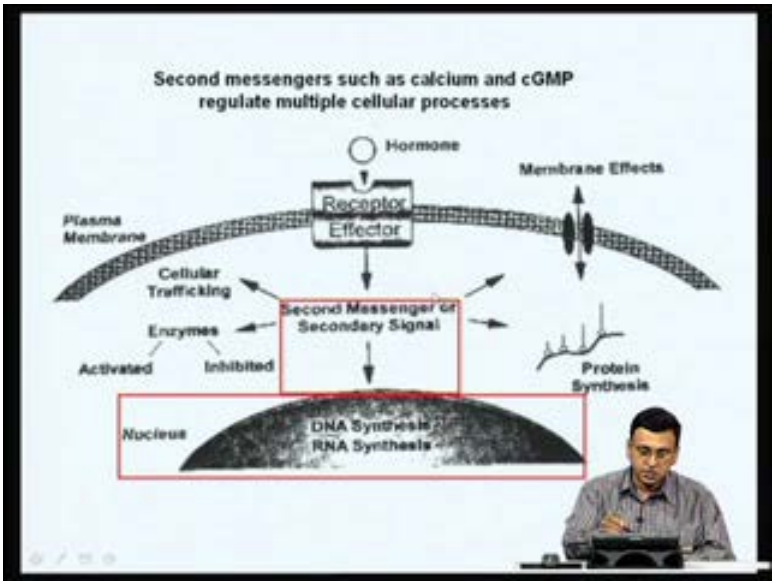


Now, what I would like to now emphasize here is that whenever we talk about second messengers like cyclic AMP calcium or cyclic GMP, they perform a number of effects. So, what I would like to emphasize from this cartoon is that second messenger such as calcium or cyclic GMP regulate multiple cellular processes and as we have shown here, they can you know of a cellular trafficking. They can alter the enzyme activity, they can either activity enzymes, they can inhibit enzymes, they can inhibit protein syntheses or they can alter ion channels. So, without info of ions can be affected, but since, in this particular course we are focusing our attention primarily on regulation of gene expression. We are not going to discuss some of these aspects. So, we are going to focus primarily on how second messengers regulate transcription inside the nuclear and therefore, we are not going to discuss many of these aspects.

So, when I say this is what second messengers do inside the cell, please be aware that second messengers also do a number of other things, but since our focus in this course is primarily on regulation of gene expression, we are not going to discuss many of these aspects. So, we are going to focus primarily on how binding of ligand to a cell surface receptor is resulting in the syntheses of second messengers and how this second messenger go and influence gene expression program. This is what we are going to focus and we are not going to discuss many other things that second messenger do outside the nucleus, but these are all very important processes and they have a very important applications in a number of biomedical brilliance and many diseases actually manifest because of these role of second messengers in the activity, in the activation on repression of number of enzymes including essiac, diabetes and so on so forth are all affected by this second messengers signaling.

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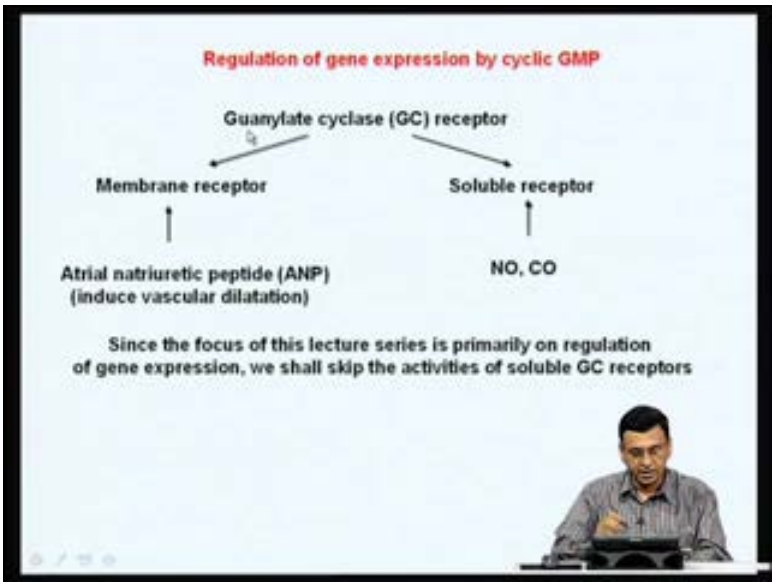


Now, let us spend some time to understand how cyclic GMP regulate gene expression. As I just mentioned when the ligand or the hormone binds to a specific GpCr, it results in the activity of G protein and these G protein, now the trimeric G protein, the alpha subunit separates and this alpha sub unit separates now goes and activates adenylate. Some of these receptors into have intrinsic Guanylate cyclase activity and as a result, these are all in the syntheses of cyclic GMP. Now, there are actually two kinds of cyclic Guanylate cyclase receptors and what we have been discussing so far is about the membrane receptor and which actually results in enzyme cyclic GMP.

There are also what are called as soluble receptors and which actually are activated by molecules like nitric oxide and carbon monoxide and so on and so forth. Now, again there is a similar example studied in the case of the protein kinase A. Protein kinase A also exist both in the form of soluble protein kinase A as well as protein kinase A and kind of anchors proteins like akap ankers protein kinases and then, keep it in a anker form and the soluble protein kinase A. Protein kinase A promote different kind of function and the anker protein kinase A perform a different kind of function. Similarly, the Guanylate cyclase also there are two types of the membrane bound Guanylate cyclase and the soluble Guanylate cyclase receptors and the molecule gaseous molecules like nitric oxide carbon monoxide primarily activate the soluble receptors where as ploy peptide hormones like the a t natriuretic peptide or A N P which actually plays a very important role in vascular dilatation.

So, as a very important role in cardiovascular disorders or regulation of blood pressure and so on and so forth, these peptides when they bind to their specific membrane receptors, they act with the membrane bound Guanylate cyclase receptors. Now, since the focus in our lecture series is primarily on the regulation of gene expression, we shall keep the activities of the soluble GCGC receptors and confine our self to how the membrane Guanylate cyclase receptors influence gene expression programs.

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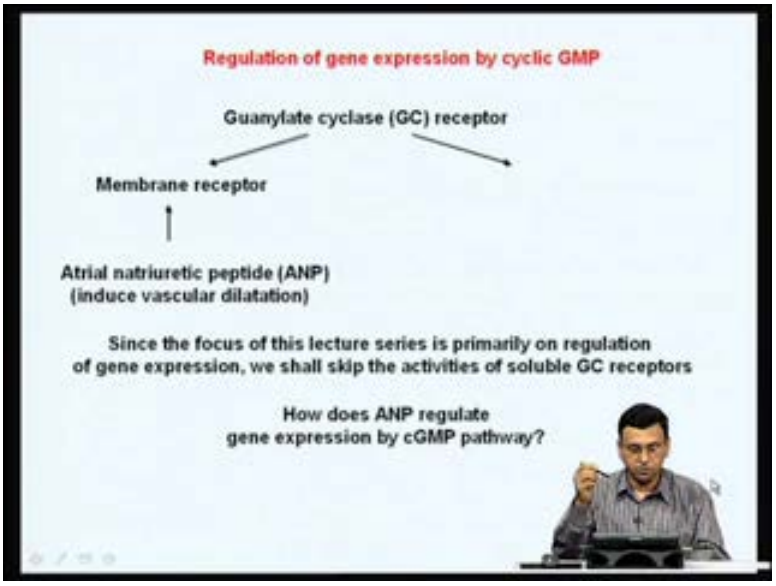

So, let us now try to understand how molecules like AMP when they intact with the Guanylate cyclase receptor, how it results in the, affects the gene expression.

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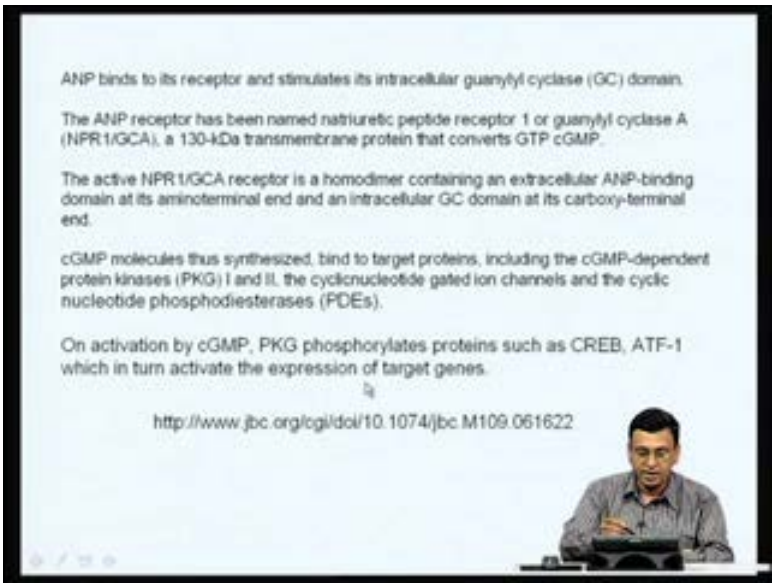


Now, ANP is a very important molecule and when it binds to its receptor, it stimulate its intra-cellular Guanylate cyclase domain. So, this is slightly different from what we have discussed so far. The earlier discussions of activation of Guanylate cyclase when the ligand binds to the receptor, it activates a primary G protein and this G protein now, the G alpha subunit goes and activates adenylate cyclase.

In the case of cyclic gene p pathway, the hormone directly binds to receptor. It has inherency Guanylate cyclase activity and this Guanylate cyclase activity is activated when the hormone binds to the Guanylate cyclase receptor and the ANP receptor has been named natriuretic peptide receptor 1 or Guanylate cyclase A which is a trans mammalian protein which is capable of converting GTP to cyclic GMP. The active GCA receptor is a homodimer containing an extracellular ANP binding domain at your amino terminal domain to which the ligand binds and an intracellular Guanylate cyclase domain at your carboxyl terminal domain.

So, once the ligand binds the domain, it causes a conformational change and as a result, the cytoplasmic Guanylate cyclase domain is activated and that now converts GMP GT GMP into cyclic GMP Gtp to cyclic GMP. Now, the cyclic GMP molecule is thus synthesized bind to specific target proteins including cyclic GMP dependent protein

kinases 1, but there are about two different kinds of kineses called PKG1, PKG2 and a cyclic nucleotide gated ion channels and the cyclic nucleotide phosphodiesterases so on and so forth. So, the cyclic GNP as a number of targets include in the kinases the gated ion channels as well as nucleotide diphosphate which actually clean this cyclic GMP back to inactivate form.

So, on activation by cyclic GMP PKG phosphorlates protein such as CREB ATF1 this in turn activates the expression of various target genes. So, this is basically the mechanism by which when a hormone like when a molecules like ANP bind to the receptors which are nothing, but Guanylate cyclases which as intensive Guanylate cyclase activity binding of this hormone to this receptor activates that Guanylate cyclese activity resulting syntheses of cyclic GMP, which then activates a specific kinases influences ion channels as well as specific phosphodiesterases.

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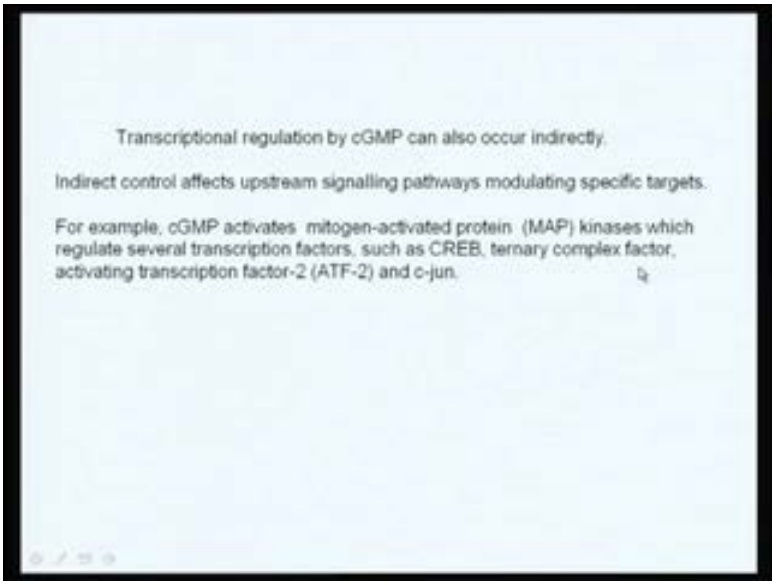


Now, transcription regulation by cyclic GMP can also occur indirectly. The indirect control affects upstream signaling pathways modulating specific targets. The previous slide we actually discussed the mechanism by which cyclic AMP directly phosphorylate activates cyclic GMP kinases or phosphodiesterases. In turn, they target transcription factors and activate or repress gene expression programs. Now, there is also indirect method by which cyclic GMP regulate gene expression. For example, cGMP can activate

mitogen activated protein kinases or map kinases which regulate several transcription factors, such as CREB, ternary complex factor 2 (ATF 2), c-jun etcetera.

So, the cyclic GMP has two different roles as far as the gene expression is concerned. In one case, the cyclic GMP activates specific kinases like cyclic GMP kinases or protein kinase G, which then phosphorylates transcription factors leading to activation or repression of transcription indirect matter. The cyclic GMP goes and affects the activity of map kinases and the map kinase in turn affect the activity of transcription factors like CREB or c-jun or ATF-2 resulting in activation of different set of genes. So, depending upon whether the cyclic GMP is activating protein kinases G or cyclic means activating map kinases. Different set of genes are activated or repression and you get different physiological effects.

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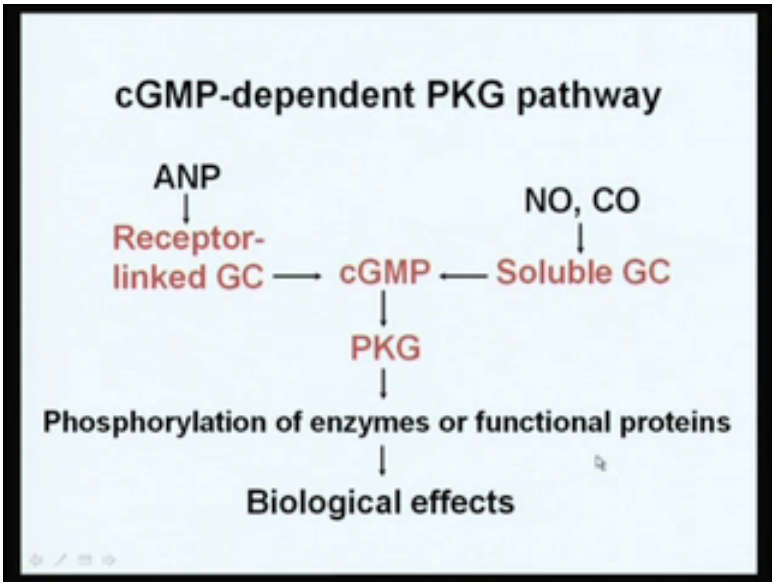


So, this is basically a summary of what we have shown discussed so far about the cyclic GMP dependent pathway. There are two different receptors where likewise we have said there are soluble Guanylate cyclases and they are receptor linked Guanylate cyclases peptide hormones like peptide molecules like. The AMP actually activate the receptor linked Guanylate cyclases leading to synthesis of cyclic GMP, whereas gracious molecules like nitric oxide and carbon monoxide, they activate this soluble Guanylate cyclases leading to synthesis of cyclic GMP and one cyclic GMP synthesis in the cells. It is in the activation of protein kinase G which goes and phosphorylates a number of

enzymes and proteins including transcription factors manifesting in the form of specific biological effects.

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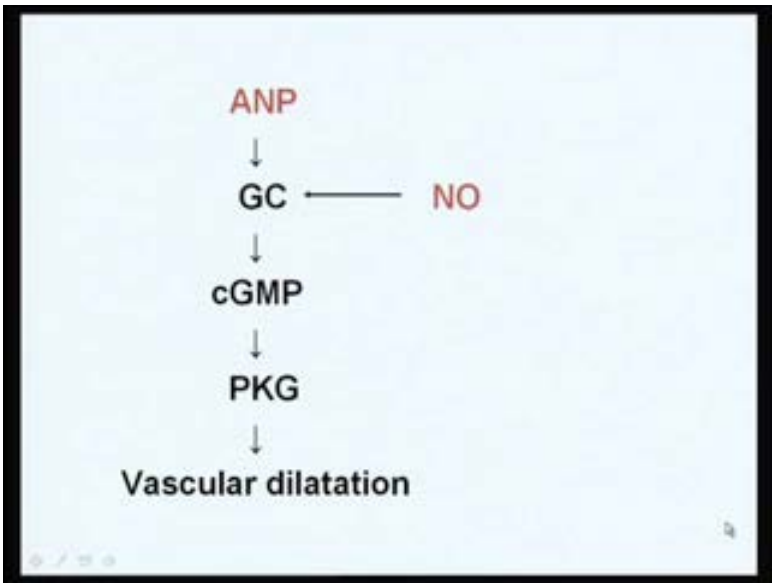


So, again once the cyclic GMP is synthesized in by the Guanylate cyclases, either by ANP or through nitric oxide, one of the major activities of cyclic GMP and protein kinase G is to induce vascular dilatation which has very important clinical effects. There are number of drugs and molecules which are actually use to cause vascular dilation and they all affect through this particular pathway. So, cyclic GMP pathway plays a very important role in vascular dilatation.

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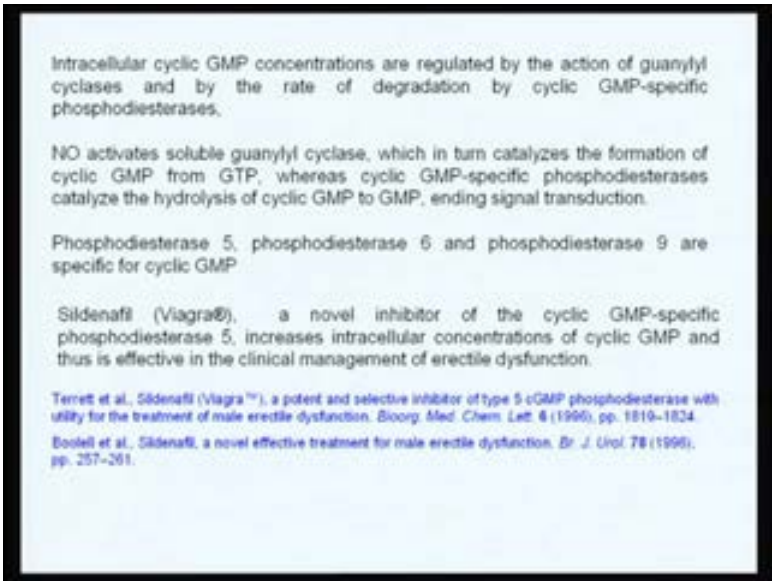


The intracellular cyclic GMP contractions are regulated by the action of Guanylate cyclases and by the rate of degradation by cyclic GMP specific phosphodiesterases. So, just as in the case of the cyclic AMP, we have seen that protein kinase A is an activated positive modulate of cyclic AMP. At the same time, the cyclic AMP is actually the protein phosphodiesterases actually inactivate the protein kinase A and then, make this cyclic AMP signaling there are phosphodiesterases the cyclic AMP and the same way the cyclic increase.

Cyclic GMP concentration are regulated both by actuation Guanylate cyclase which syntheses increase cyclic GMP concentration, whereas one cyclic GMP synthesized, they are degraded by cyclic GMP specific phosphodiesterases. I am emphasizing this point very important because not only the adenylate Guanylate cyclases which increase cyclic GMP concentrations, the GMP specific phosphodiesterases also has a very important physiological function. For example, nitric oxide activities soluble Guanylate cyclase which in turn catalyze the formation of cyclic GMP from GTP, whereas cyclic GMP specific phosphodiesterases catalyze the hydrolysis of cyclic GMP to GMP, ending the signal transduction.

So, on one hand when you activate the Guanylate cyclase with the intracellular, the concentration of cyclic GMP increases. On the other hand, when you activate a phosphodiesterases, it cleans the cyclic G and P leading to a decrease in the cyclic GMP

concentration and both have very important physiological functions. I will give one example. For example, phosphodiesterases 5 and phosphodiesterases 6 as well as phosphodiesterases 9 are very specific for cyclic GMP and specifically, cleave this cyclic GMP and in activate it. These as very important applications because one of the drugs, they known as the Sildenafil or very popularly known as Viagra which is a, it is actually novel inhibitor of cyclic GMP specific phosphodiesterases is 5 and as a result, it increases concentration of cyclic GMP. Therefore, this is very effectively used in the clinical manager management of disease like erectile dysfunction.

So, you can see inhibitors of the specific GMP phosphodiesterases can be used as very important drug molecules for treatment of specific physiological conditions. There are actually two specific papers which have coated here which actually lead to the discovery of this Viagra and then, discussed how it acts as inhibit of this phosphodiesterases. As a result, the cyclic GMP levels increases leading to specific physiological effects.

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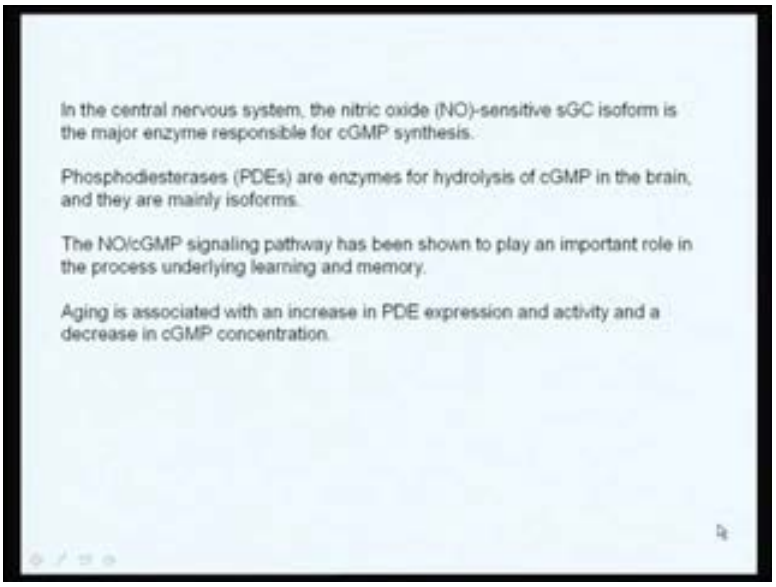


Now, in the central nervous system, the nitric oxide sensitive soluble Guanylate cyclase as form is the major enzyme required responsible for cyclic GMP synthesis. So, the soluble Guanylate cyclase plays a very important role in the nervous system and it has a very important function and phosphodiesterases catalyze the hydrolysis of cyclic GMP in the brain. There are mainly three forms which have shown in the previous slide.

So, the nitric oxide cGMP signaling pathway has been shown to play very important role in the processes underlying learning and memory. Some of these things also involve activation or repression of specific genes. Similarly, aging is associated with increasing phosphodiesterases expression and activity and as a result, a decrease in the cGMP concentration. So, in the nervous system, the cGMP plays a very important role activating a number of physiological processes, especially it is a soluble Guanylate cyclase play a very important role.

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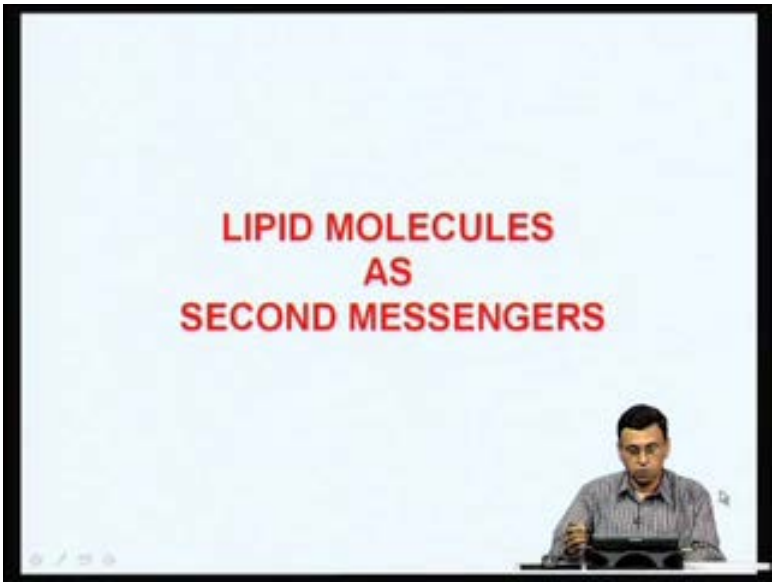


So, having discussed two important signaling molecules, namely the cyclic AMP and cyclic GMP, let us spend some time to understand how lipid molecules, such as the iniostal, triphosphate, diacylglycerol, they serve as important second messengers.

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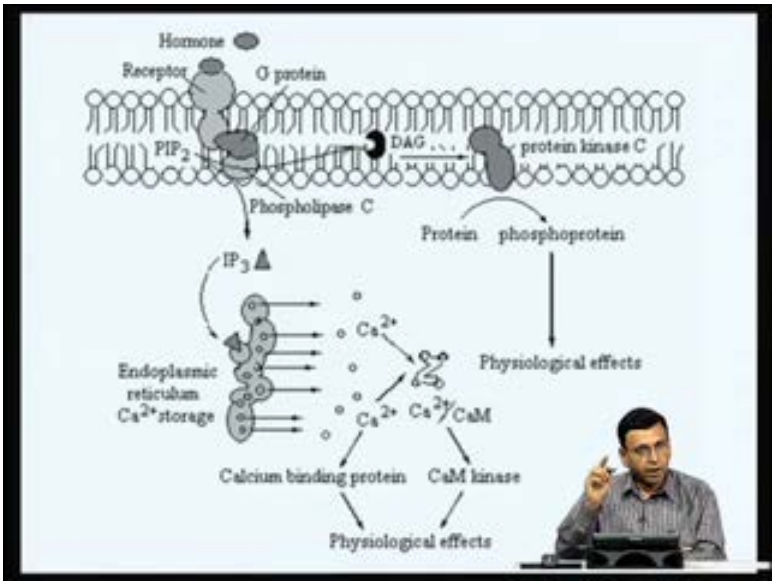


We have already discussed, but I would like to reemphasize again because these are very important second messengers like we discussed earlier. When a hormone binds to the cognate receptor, it results the activation of a specific G proteins and this trimeric G proteins, now the alpha subunit, now activates phospholipase C and as a result, the phosphatidylinositol bisphosphate is now converted into inositol triphosphate as well as the diacylglycerol.

So, PIP2 is converted to diacylglycerol and IP3 by phospholipase C and this diacylglycerol now goes and activate protein kinase A along with calcium. Now, once IP3 is synthesized or the inositol triphosphate synthesized inositol triphosphate goes and interacts with specific receptors, endoplasmic, reticulum and as a result, calcium is released from the endoplasmic reticulum. Therefore, cytosolic calcium levels raised and calcium can either go and activate together with diacylglycerol can activate protein kinase C or it can also bind to calmodulin and the calcium calmodulin complex now goes and activates cam kinases leading to specific physiological effects.

So, the two important lipid molecules which plays a very important role in the essential pathway are the diacylglycerol and the inositol triphosphate. So, these two are very important second messengers.

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Hydrolysis of just as I mentioned hydrolysis of phosphatidylinositol, bisphosphate generates diacylglycerol, inositol triphosphate and diacylglycerol together with calcium activates protein kinase C, whereas ions 2 try phosphate is required in the mobilization of or increases the intra-cellular calcium. So, these are the two major functions of these few important lipid molecules.

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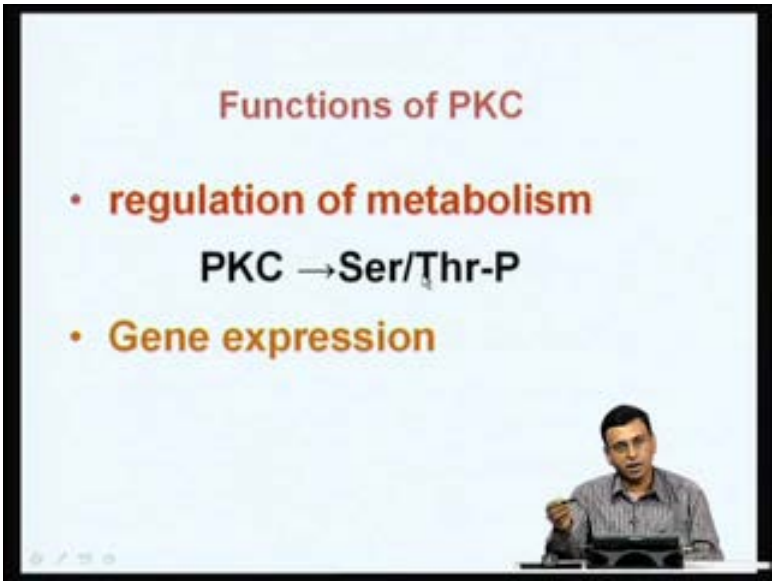


Now, what does protein kinase C do? Protein kinase C is again a very important regulator of number of metabolic processes and protein kinase C is just like we have the calcium calmodulin kinase as well as protein kinase A. Protein kinase A is also a serine threonine protein kinase. So, it goes and phosphorylates specific serine or threonine residues of a number of target proteins and not only the PKC regulates a number of enzymes involving in various metabolic pathways, the P K C also plays a very important role in activation or repression of specific gene expression programs.

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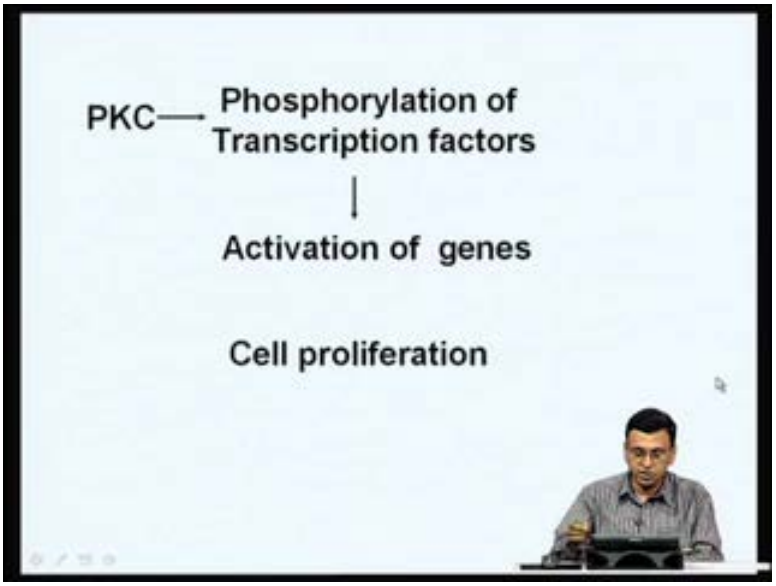

So, PKC phosphorylates number of transcription factors and one of the important transcription factors are phosphorylated by PKC is c-fos and c-jun which together are called as the AP1 or activator protein 1, but in the next class, we are going to spend a lot of time to understand how phosphorylation of these transcription factor by the protein kinase C results in the activation of very important physiological pass including cell proliferation and growth.

So, just remember, today's class that PKC plays a very important role in the phosphorylation of number of transcription factors, which results in the activation of number specific genes and as a result, activation of these genes results in proliferation of cell proliferation.

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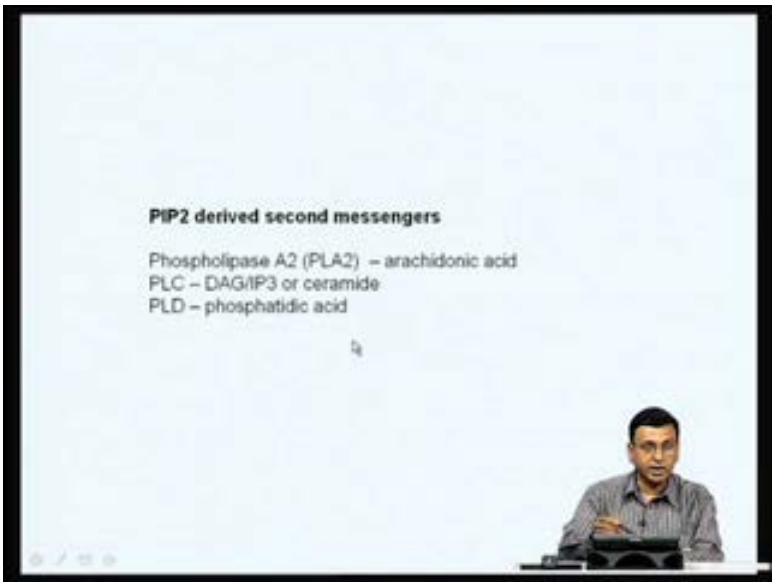


Now, in addition to this, there are also number of other lipid molecules which serve as a second messengers including for example, phospholipase A2 which actually results in the synthesis of arachidonic acid which again plays a very important role especially in a number of pathways involving processor gladdines so on and so forth. Just now we discussed that phospholipase C synthesis diacylglycerol and inositol phosphate and can also synthesis of ceramides, whereas phospholipase D involved in the synthesis of phosphatidic acid.

So, the phosphatidylinositol, bisphosphate can lead to this synthesis of number of lipid base second messenger molecules. What do I discuss so far is how phospholipase C

converts phosphatidylinositol bisphosphate to diacylglycerol and inositol triphosphate. The same phosphatidylinositol bisphosphate can also be acted upon by phospholipase A-2 resulting in the synthesis of arachidonic acid, whereas phospholipase D converts phosphatidylinositol bisphosphate to phosphatidic acid. All these things has very important physiological functions.

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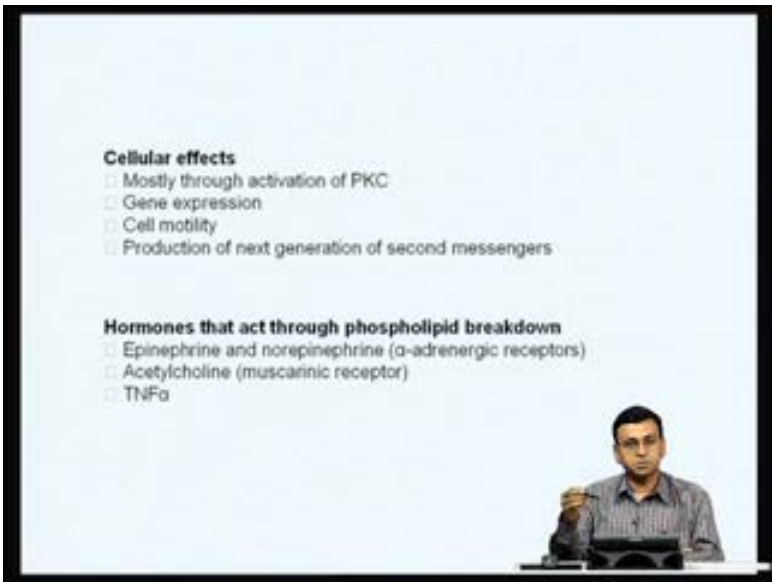


So, what are the cellular effects of some of these second messengers protein kinase C. Some of these lipid molecules primarily activate the protein kinase C and protein kinase C as I just mentioned plays a very important role in the alteration of specific gene expression programs. In addition, it also number of other processes which does not involve activation or repression of gene expression, such as cell mobility as well as production of next generation of second messengers.

A number of hormones actually act through this phospholipid breakdown. So, when you have this phosphatidylinositol molecules, these phosphatidyl molecules which are present in cell membrane are now precocious for a number of second messengers and when hormone like epinephrine, norepinephrine buying to alpha adrenergic receptors or acetylcholine binds to muscarinic receptors or when TNFa of alpha tumor necrosis factor alpha binds to its TNFa of receptor. All these things ultimately results in the breakdown of phospholipids, such as phosphatidylinositol bisphosphate resulting in the generation of either activation of phospholipase A-2 or protein kinase C or phospholipase d resulting

synthesis of specific second messengers molecules. All these second messenger molecules have a very important physiological functions and protein kinase C especially plays a very important role in the activation of transcription factors like CREB, like c-jun, c-fos etcetera leading to cell proliferation.

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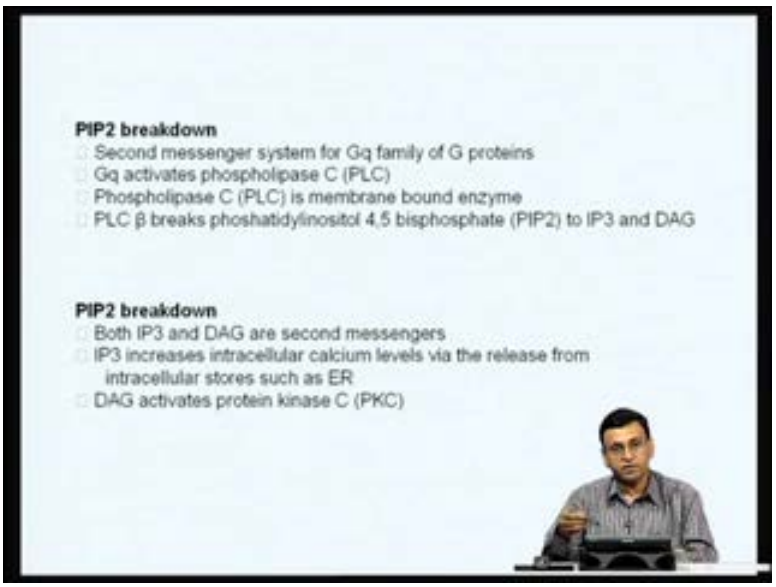


Now the phosphatidylinositol bisphosphate breakdown is the second messenger system for the Gq family of G proteins. So, as I mentioned in the cartoon, the Gq is the G alpha sub unit and Gq actually specifically activates phospholipase C, where as the Gs sub unit specifically activates the adenylate cyclase leading to sub cyclic AMP. So, there are hormone molecules which interact with specific set of G proteins and which contains G alpha Gs subunit and this Gs subunit interacts with the adenylate cyclase leading to the activation of synthesis of cyclic KMP.

Where as, in the case of the lipid molecule second messenger system, the Gq family of G proteins, where the alpha subunit is called as Gq and these Gq instead of interactive with adenylate cyclase actually interacts with phospholipase C, activates phospholipase C resulting the conversion of breakdown of phosphatidylinositol bisphosphate into diacylglycerol and inositol triphosphate. So, Gq actually activates phospholipase C and the phospholipase C is a membrane bound enzyme and phospholipase C breaks down this phosphatidylinositol bisphosphate into inositol triphosphate diacylglycerol.

So, you can see there are two different kinds of G proteins. One kind of G proteins containing the Gs subunit and it interact to the adenylate cyclase results in the synthesis of cyclic AMP. Another set of G proteins containing the Gq subunit. The G the alpha subunit is called as Gq which interact this phospholipase C resulted in the breakdown of inositol bisphosphate into inositol triphosphate and diacylglycerol which act as very important cycling molecules.

Now, the point that we would like to emphasize is that the breakdown of phosphatidylinositol bisphosphate results in generation of IP3 and IS glycerol both act as second messengers while inositol tri inositol triphosphate increases intra-cellular levels of calcium resulting in the region from intra-cellular resources of endoplasmic reticulum diacylglycerol primarily activates protein kinase C and protein kinase C in turn goes and activates a number of phosphorylates, a number of transcription factors and metabolic enzyme bringing about specific biological effects.

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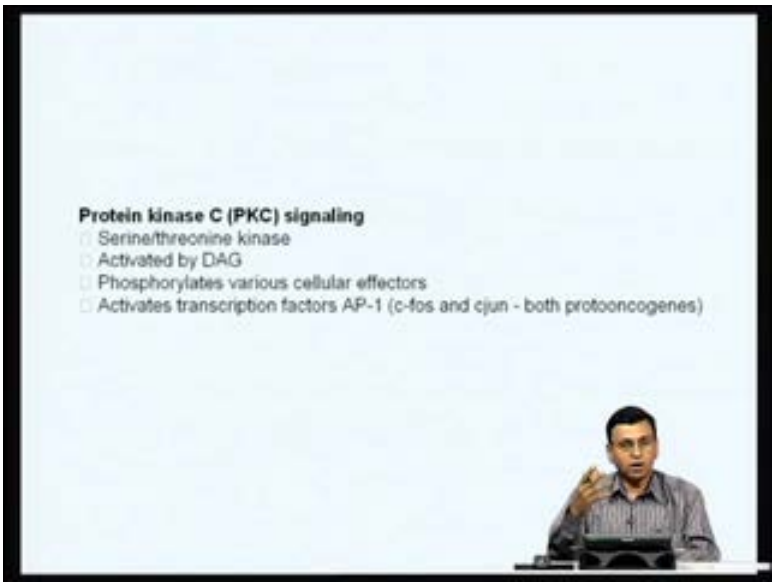


So, the protein kinase C signaling is a very important signaling pathway. So, we are going to spend considerable amount of time in the next few, in the next class to understand what are the major transcription factors which are phosphorylates by protein kinase C and how phosphorylation of this particular transcription factors results in the activation or repression of specific gene expression and how this manifest in the form of specific physiological effects.

So, protein kinase C is a serine threonine kinase. It is activated by diacylglycerol as well as calcium and it phosphorylates various cellular effects. One of the major targets for protein kinase C signaling pathway is the transcription factors AP-1 which consists of c-fos and c-jun and both are protooncogenes. The levels of c-foes and c-jun are very finely controlled inside the cells and if you have, if you over express some of these proteins, it can result in cancer.

So, the PKC signaling pathway plays a very important role. For example, if you treat cells with molecules like phorbolesters which activate the protein kinase C resulting in the synthesis of diacylglycerol and sorry, resulting in the activation of AP-1, it can result in cancer. So, in fact, the PKC activation is a very important animal model for understanding cancer, where if you treat mice for example, continuously with molecules like phorbolesters, you can actually demonstrate the development of tumor right at the sight of treatment. So, you can actually induce tumors in mice by simply treating with phorbolesters which actually like TPA tetra forebrain for oil technic acid which actually can cause activation of protein kinase C resulting in the activation of AP-1 are relating to cancer. So, PKC signaling plays a very important role in cell proliferation and abnormal activation of PKC signaling can convert normal cell into cancer cells.

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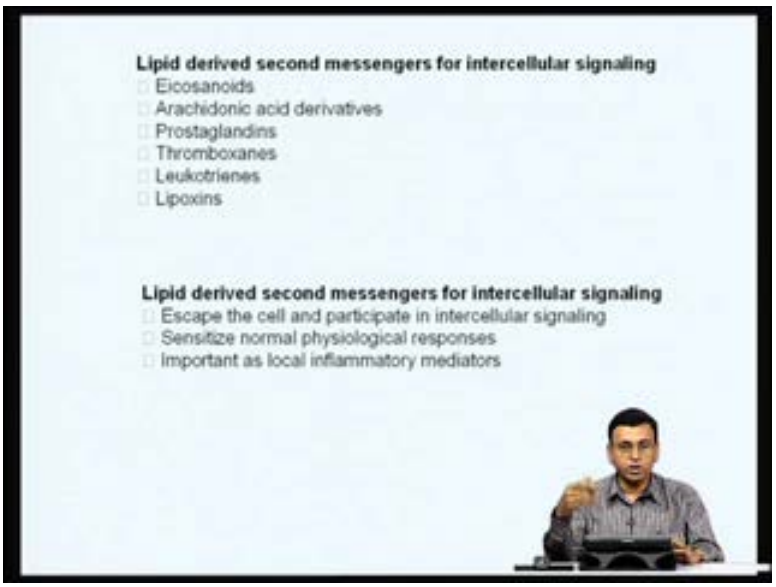


In addition to the diacylglycerol and inositol triphosphate which we have discussed just now, there are number of other lipid molecules which actually serve as second

messengers for inter-cellular signaling. The IP3 and diacylglycerol we have discussed so far basically serves as molecules second messengers for intra-cellular signaling. A number of lipid molecules also serve as second messengers for inter-cellular signaling. Some of them are listed here. This includes eicosanoids, arachidonic acid derivatives, prostaglandins, thromboxanes, leukotrienes and lipoxins and again all these molecules are very important from the biomedical point of view. They all control very important physiological processes and a number of pharmaceutical companies are trying to develop molecules which either antagonize or which can stimulate the action of this block. The action of the second messengers, there we have very important role in process like inflammation, controlling pain and so on and so forth.

So, these pathways, second messenger pathway involving lipid molecules has tremendous pharmalogical importance and all these molecules are very important from the pharmalogical point of view and a number of molecules which are available in the market either act as block. The pathways involving the second messengers or activate the pathways involving the second messengers leading to specific clinical benefits.

So, the lipid derive the second messengers for inter-cellular signaling. They actually, once these second messengers in the cell, they escape from the cell where they are synthesized and participate in specific inter-cellular signaling, they synthesize normal physiological responses and they act as important local informatory mediators. So, you can see activation of when activation of these lipid base second messengers has a very important role not only in controlling a number of physiological processes, but also for a number of therapeutic benefits. So, a number of drug molecules which either block or activate these second messenger pathways are used routinely in a number of processes like anti-inflammation agents and in pain management and so on and so forth.

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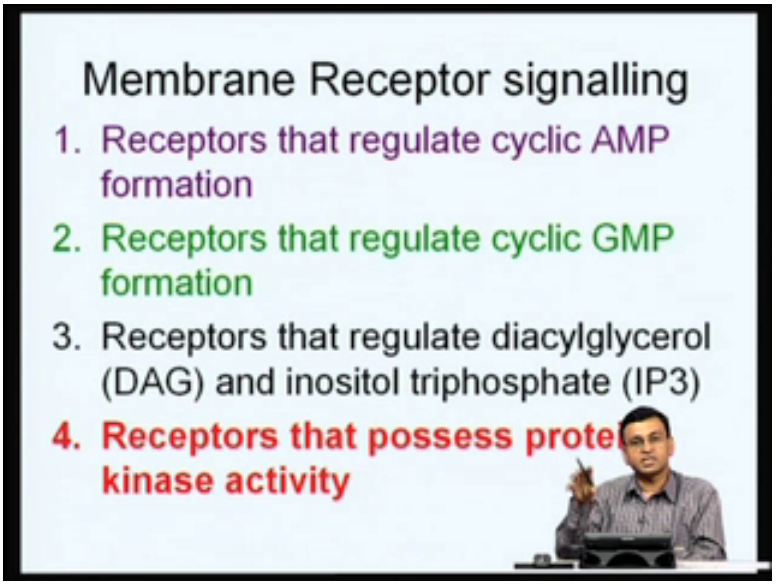


So, what we have discussed so far in the last 2 classes, last 3 classes basically, we started discussing about how cell, how genes which are present inside the nucleus where there expression has to be regulated by molecules which are present in the outside and we discussed primarily how molecules which cannot enter the cell, especially the hydrophilic molecules or water soluble compounds, how when they interact with specific membrane receptors can activate specific kinases leading to the synthesis specific second messenger molecules. These second messenger molecules in turn activates specific protein kinases and they in turn phosphorylates specific transcription factors leading to activation or repression of target genes.

So, for among the various membrane receptors, we focused our attention primarily on one class of receptors called as the GPCRs or G protein coupled receptors and these G protein coupled receptor are characterized by the presence of the serpentine receptors or the 7 trans membrane helices. They act as receptors for a number of very important hormones like epinephrine, norfenefrine and so on and so forth and we discussed very clearly when these G proteins are activated, the G alpha sub unit get disassociated.

In one case, these G alpha sub unit goes and activates adenylate cyclase leading to this syntheses of cyclic AMP. So, we have one class of receptors that regulates cyclic AMP formation. The G sub unit of these are called as the Gs subunits and there are also other kind of receptors which have an intrinsic cyclic adenylate guanylate activity, guanylate

cyclase activity. When these receptors are activated by molecules like ANF, AMP or atrial natriuretic peptide AMP, the cyclic JMP synthesize because of the activation of the guanylate cyclase activity of these receptors and this cyclic GMP in turn can activate a number of physiological processes.

Now, what we have also discussed today is that there are another kind of receptors which can activate a specific set of G proteins in which the genes Ga G alpha subunit in this case it is called the Gq sub unit. Now, just as the Gs subunit activates adenylate cyclase, the Gq subunit goes and activates the phospholipase C and activation of the phospholipase C by these kind of genes, class of G proteins now results in the syntheses of diacylglycerol and inositol triphosphate from molecules like phosphatidylinositol, bisphosphate and this diacylglycerol and inositol triphosphate that is synthesized, now act as very important second messengers.

So, basically we have discussed so far about three different classes of G protein coupled receptors. In one case, the activation of these G proteins results in the synthesis of cyclic AMP. In another case, it results in the activation of cyclic GMP and in one more case, it results in the synthesis of two importance of second messengers, namely diacylglycerol and inositol triphosphate. Now, what we have to discuss today is that GPCR are not the only class of membrane receptors. There are also receptors which possess intrinsic protein kinase activity.

There are receptors which have intrinsic tyrosine kinase activity and there are also receptors which when bound by their higher and activate or recruit tyrosine kinase or protein kinases. So, we have to now discuss another class of membrane receptors which have intrinsic protein kinase activity or which can actually recruit protein kinases. As a result, a different kind of phosphorylation cascade is initiated leading to an activation or repression of target genes. Now, before we discuss how tyrosine kinases act as very important signal transuding molecules, especially for molecules like growth factors and many of the growth factors like the EGF epidermal growth factor receptor, they possess intrinsic tyrosine kinase activity.

We have to now discuss how molecules like growth factors, when the epidermal growth factor when they bind to specific receptors, the tyrosine kinase activity of this receptor is activated and as a result, a different kind of G proteins, in this case monomeric G

proteins, especially the rat family of proteins, they get activated. Again, you have initiation of different kind of a map kinase cascade leading to activation or repression of trans target genes.

So, the activation of the tyrosine kinases involve activation of monomer G proteins, such as, but so far our discussion has been primarily confined, especially with the GPCRs. We have confined our discussion primarily to G proteins which involve trimeric G proteins and these trimeric G proteins are involved either in the syntheses of cyclic AMP or in the syntheses of diacylglycerol and inositol triphosphate.

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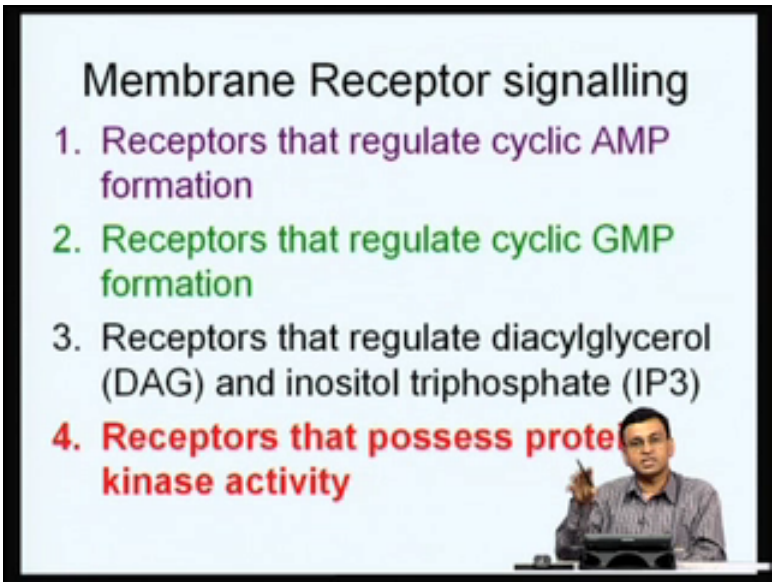


So, what we will discuss in the next few classes is, when certain hormones especially the polypeptide growth factors like epidermal growth factor, when they bind to the cells surface receptors, these cells surface receptors tyrosine kinase activity is activated and as a result, it now activates monomeric G proteins like ras and this ras in turn activates a series of kinases leading to the activation of the map kinases pathway resulting in the activation of specific target genes.

Before we now go and discuss into tyrosine signaling pathway, I would like to discuss in the next class how the protein kinases C which is what is the crux of the today's discussion, how once protein kinase C is synthesized or activated in the cells, how protein kinase C goes and activates specific transcription factors. Especially we are going to discuss how protein kinase C activates what are known as the early response as well as

late response genes and protein kinase C goes and phosphorates proteins like activates molecules like c-fos and c-jun and this c-fos and c-jun are called the immediate response genes or the early response genes. These c-fos and c-jun again gets phosphorylate by protein kinase C and this in turn go and activate transcription parallel. Again, NF Kappa B which are called as the late response genes and these NF Kappa B, then goes and ultimately results in the proliferation of cells.

So, the entire cell proliferation program can be activated by the activation of protein kinase C. What we will discuss in next class is how protein kinase C can regulate gene expression by activation of both genes involved in the early response as well as late response and how transcription factor, such as c-fos and c-jun can in turn activate the genes involved in late response leading to cell proliferation. This protein kinase C signaling pathway is very important because other end expression of this transcription factor and other end activation of protein kinase C can result in cancer.

So, we need to understand how protein kinase C activity is regulated inside the cell, what kind of molecules actually activate protein kinase C and what kind of transcription factors are involved in the activation of cell proliferation program and how the entire process is regulated and how protein kinase C plays a very important role in control of cell growth and cell differentiation.

We will also discuss in very detail the mechanism by which transcription factors have c-fos and c-jun bind DNA and what kind of transcription activation domain they actually posses and what kind of residues are phosphorylated by protein kinases C when these phosphorylation in trans activation domains, when these serine threonine residues of c-fos or c-jun phosphorylated, it can result in the activation of transcription. The very interesting thing that we are going to discuss about AP1 is that the c-fos and c-jun, both of them contained these basic region leucine zipper proteins and thus, is very similar to the CREB which is involved in the cyclic AMP response.

So, just as protein kinase A activates CREB, which is a leucine zipper protein resulting in the activation of cyclic AMP responsive genes in the case of protein kinase C. Protein kinase C phosphorylates proteins like c-fos and c-jun and as a result, c-fos and c-jun dimerise and these dimers now go and bind to specific response elements which of genes are involved in cell proliferation. A very interesting thing I want to point out is that the

cyclic AMP response element which is the bind bound by the CREB protein and the response element to with the protein kinase C activated AP1 or c-fos and c-jun bind. They are very similar.

The sequence to which CREB binds is DGACGTCA whereas, the sequence to which the AP1 binds is TGACTCA, there is just one nucleotide which is difference between AP1 binding side and a CREB binding side, but one CREB gets activated by the protein kinase A pathway. You have a totally different kind of physical response if you activate protein kinase A. If you activate the AP1 through the protein kinase C pathway, you have different kinds of signaling response.

So, you can see there are two independent signal transduction pathways. One is protein kinase A mediated, another is protein kinase C mediated. Protein kinase A activates CREB, protein kinase C activates AP1, but ultimately at the DNA level if you look at the sequence to which these two different kinds of transcription factors bind, there is only one nucleotide which distinguishes these two elements. CREB binds TGACGTCA whereas, AP1 binds TGACTCA.

Instead of CGTCA, you have CTCA. So, just one nucleotide difference can bring about totally two different physiological responses. In one case, it activates cyclic AMP response, another case it lead to a cell proliferation. So, although you have molecules which are totally diverse at the cellular level, at the molecular level, you see single nucleotide or single amino acid differences can bring about totally different physiological responses and result in an activation or repression of totally different kind of enzymes and proteins and so on so forth.

So, let us now try to understand in the next class, once protein kinase C is activated, what kind of transcription factors are phosphorylated into protein kinase C and what kind of physiological response is brought about by protein kinase C activation. Once we finish that, we will go back and discuss a different kind of membrane receptors, namely those membrane receptors are which process intrinsic tyrosine kinase activity and how these tyrosine kinase receptors transduce signals through monomeric G proteins like ras leading to activation or repression of specific target genes. I think I will stop here.