Eukaryotic Gene Expression: Basics and Benefits
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Lecture No. # 18

Regulation of Gene Expression by Protein Kinase C

We will continue our discussion about the regulation of gene expression by G-protein coupled receptors.

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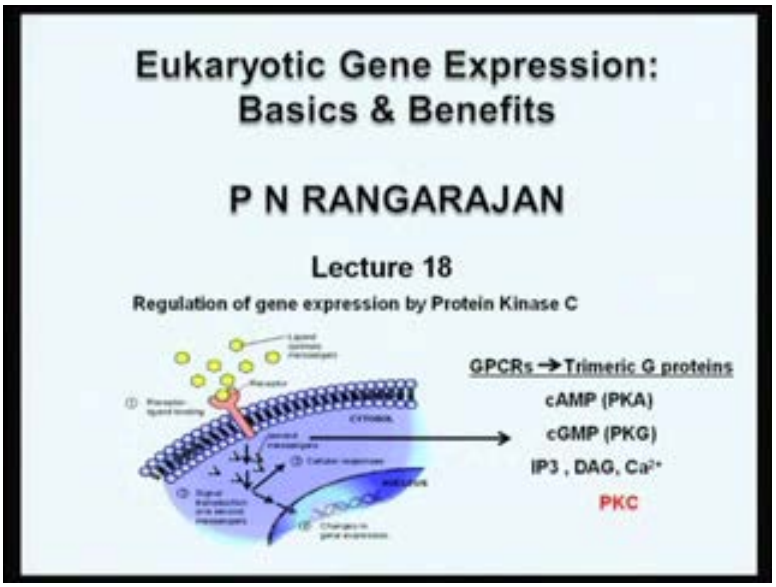

This is what we have been discussing in the last three classes: how signaling molecules, when they interact with specific receptors, this, specifically the serpentine receptors of the seven trans membrane domain receptors leading to the… lead result in the activation of trimeric G-proteins, and this results in dissociation of the beta, gamma, and alpha subunits, and they then interact with specific effector molecules like either adenylate cyclase or phospholipase C, resulting in second generation of specific second messengers, and these second messengers are, ultimately go and then phosphorylate activates specific kinases, resulting in activation or repression of specific genes, as well as activation or repression of metabolism enzymes, ion channels, and so on and so forth.

What we are discussed in the last three classes is, how signaling molecules interact with G-protein coupled receptors. This results in the activation of trimeric G-proteins, and we discussed primarily about those G-proteins, which, ultimately, activate protein kinase A

signaling, protein kinase G signaling. And in the last class, we discussed, basically, about the generation of second messenger such as inositol triphosphate, diacylglycerol, calcium, and how these three molecules, when they are generated by specific signaling molecules, results in the activation of protein kinase C as well as calcium calmodulin kinase, and so on and so forth.

So, what will do today is, will going to spend considerable amount of time to discuss about protein kinase C, because it is very, very important protein kinase, and we will discuss about how protein kinase is generated by varying signaling molecules through G-protein couple receptor pathway, and what kind of… we will give take some– discuss– some examples of gene– genes or gene expression pathways, which are activated by protein kinase C.

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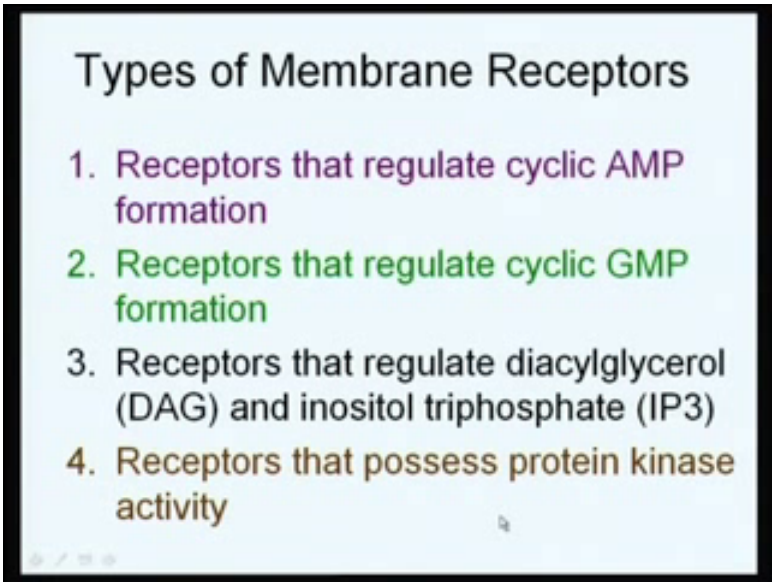

So, we have been discussing, primarily, about membrane receptors, and how ligands and hormones, when they bind to these membrane receptors, lead to the activation of specific signaling pathways, ultimately culminating in activation or repression of genes. So far, we have discussed about receptors that regulate cyclic AMP formation; receptors that regulates cyclic GMP formation. Today, we are going to spend some time about the receptors that regulate diacylglycerol and inositol triphosphate formation; how these molecules, in turn, regulate gene expression, and maybe in the next class, we are going discuss about receptors that possess protein kinase activity.

So, since we have already covered the first two, today, we are going to, finally, focus on third kind of membrane receptors, namely, those receptors which activates G-proteins that ultimately result in activation of phospholipase C, resulting the generation of diacylglycerol and inositol triphosphate, and how these act as signaling molecules.

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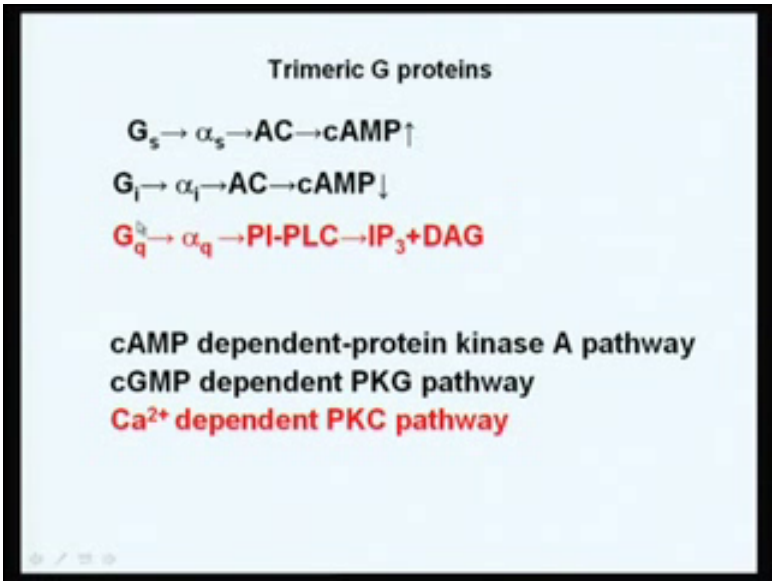

As I just mentioned, of the various trimeric G-proteins that we have been discussing, so far, we have discussed about the G s alpha subunit, which primarily activates adenylate cyclase, leading to increase in cyclic AMP. There are also molecules which activates G i– G alpha i– subunit, resulting in the down regulation of cyclic GMP by actually activating phosphodiesterases, and we also then discussed about the cyclic GMP pathway, where the membrane receptors itself contains intrinsic cyclic guanylate cyclase activity. This results in the synthesis of cyclic GMP, and then how cyclic GMP regulates gene expression, especially in calcium depending manner.

Today, we are primarily going to focus about trimeric G-proteins, containing G q– G alpha q subunit, and when they are activated, how they activate phospholipase C, so that it results in generation of inositol triphaosphate and diacylglycerol. We have already discussed in a last class quite a bit about this particular aspect, but we will just briefly go through what we have discussed, and then get into the mechanism by which protein kinase C act as a important activator of various gene expression pathways.

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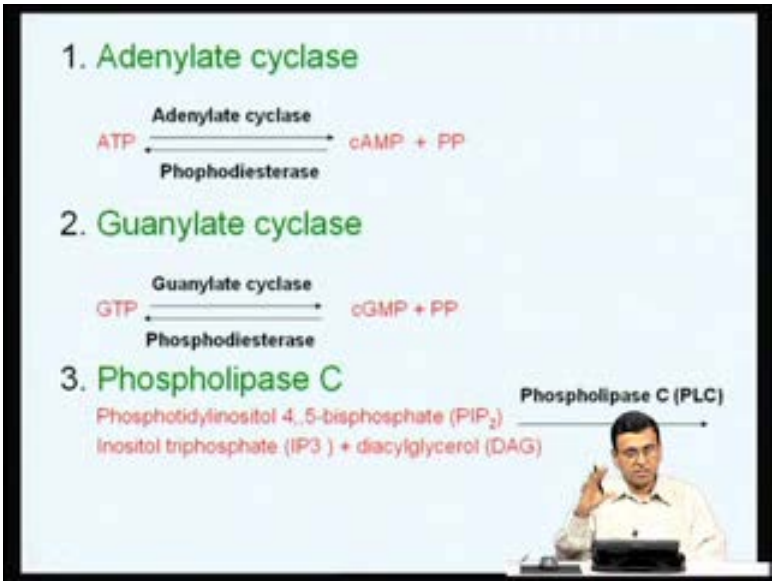

As I said, when we talk about the membrane receptors, especially G-protein coupled receptors, there are three major enzymes that we have to remember– one is the adenylate cyclase, which I have already discussed, which basically converts ATP to cyclic AMP and pyrophosphate, and cyclic AMP also is converted that by phophodiesterase to ATP, and this is of reverse of signaling.

Regulation takes place both by cyclic AMP generation as well as by cyclic AMP cleavage by phophodiesterases. We also discussed about guanylate cyclase, which again, a very important signaling pathway; how GTP is converted to cGMP, and then again how a cGMP is converted back to GTP by phophodiesterases, and how these signaling events ultimately result in activation and repression of transcription.

So, today, we are going to focus about the third important enzyme in GPC or signaling pathway, namely the phospholipase C– phospholipase C– or the PLC, which basically converts phosphatidylinositol 4,5 bisphosphate, or PIP 2, to inositol triphosphate or IP 3 and diacylglycerol or DAG. So, the focus today is going to be about this particular aspect.

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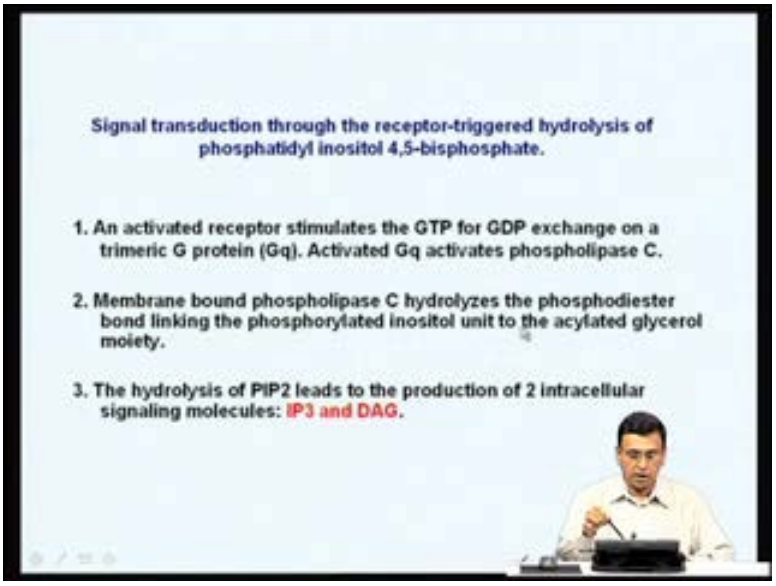

So, signal transduction through the receptor triggered hydrolysis of phophatidylinositol 4,5-bisphosphate primarily happens when a receptor, which is activated by a signaling molecule– it could be a ligand; it could be a small molecule or it could be a polypeptide hormone– that results in the stimulation of GTP to GDP exchange on a trimeric G-protein, and those G-proteins which activate phospholipase C– the alpha subunit– of our referred to as G q and alpha subunit also refer is to as G alpha q subunit, and the G q actually activates phospholipase C.

So, when this activation takes place, the membrane-bound phospholipase C hydrolyzes the phosphodiester bond linking the phosphatidyl inositol unit to the acylated glycerol moiety, and as a result of this hydrolysis, it results in the generation of two very important intracellular molecules, namely, IP 3 and DAG; that is, inositol triphosphate and diacylglycerol.

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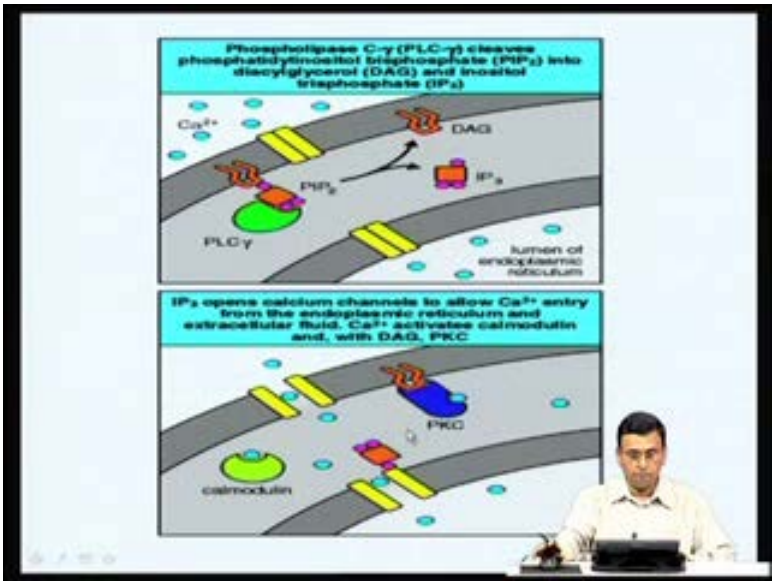

This is a schematic basically explains what we are going to discuss today, and what have been discussing so far. Basically, when the G-protein gets activated, the G-protein basically activates the phospholipase C, and when the phospholipase C is activated, the PIP 2, that is, phosphatidylinositol bisphosphate, is converted to diacylglycerol and inositol triphosphate, and the inositol triphosphate then interact with specific receptors on the endoplasmic reticulum, and as a result, the calcium, which is present inside endoplasm reticulum, is now released and the calcium thus released, now, along with the diacylglycerol, activates protein kinase C, and this activated protein kinase C now goes and activates– phosporylates– a number of target proteins, which could be metabolic enzymes, ion channel, proteins, or transcription factors.

But as I have been telling you all the time, these second messengers molecules– whether it can be cyclic AMP or cyclic GMP, calcium or IP 3 or diacylglycerol– they perform pleiotropic functions, but in this lecture series we are going to focus primarily on how these second messengers ultimately activate the regulation of gene expression. So, we are not going to talk too much about activation or repression of metabolic enzymes or activation of expression of ion channels by these second messenger molecules, although they are very, very important from the biology and medical point of view.

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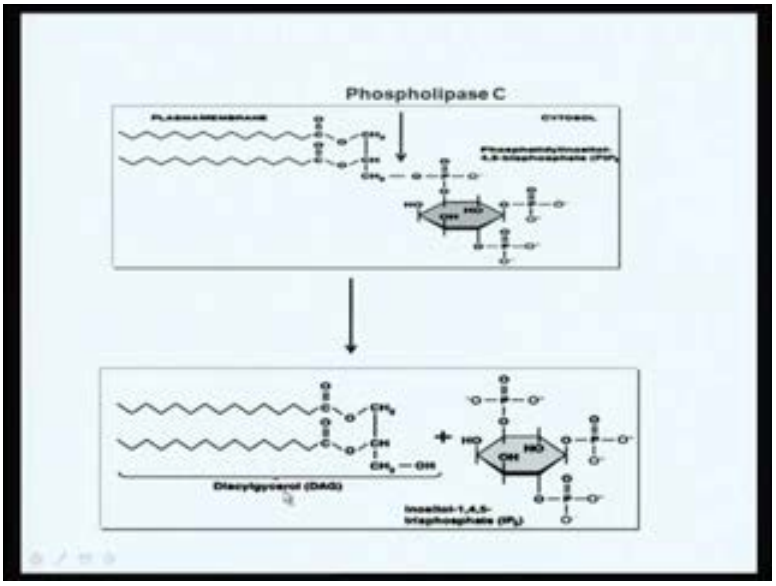

So, this is the structure of the phosphatidylinositol 4,5-bisphosphate, and when this PIP 2 is cleaned by phospholipase C, basically results in generation of inositol triphosphate and diacylglycerol, as I shown here, which are the two signaling molecules, and how they are going to act is what we are going to discuss today. So, phospholipase C actually cleaves this particular phosphodiester bond. As a result, hydrolysis of this bond results in generation of diacylglycerol and inositol triphosphate. We know inositol 1,2,3 phosphate, and this is the diacylglycerol, where we have the glycerol backbone link to… to fatty acid chains. So, diacylglycerol….

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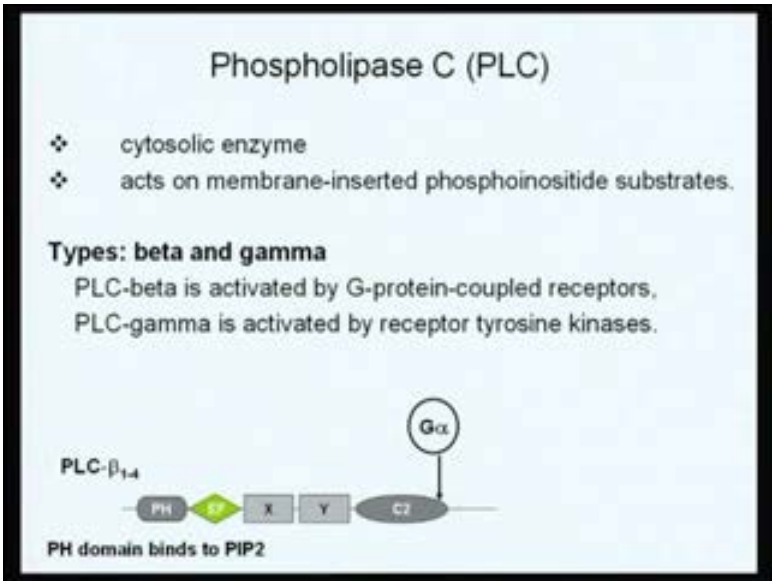

Now phospholipase C is a cytosolic enzyme. It acts on membrane-inserted phosphoinositide substrates. There are actually two types of phospholipases– one is phospholipase C beta, which is activated by G-protein coupled receptors– this is what we are going to discuss today. There is also another phospholipase C, which is called phospholipase C gamma, which is primarily activated by receptor tyrosine kinases, which we are going to discuss, maybe in the next or the coming classes– in the next few classes. So, this is basically the various structural domains of the phospholipase C beta, which contains various domains, as well from regulatory point of view, it is the C 2 domain which actually interacts the G alpha for the subunit of the GTP is, and the P H domain actually bind to the PIP 2– phophatidtylinositol bisphosphate

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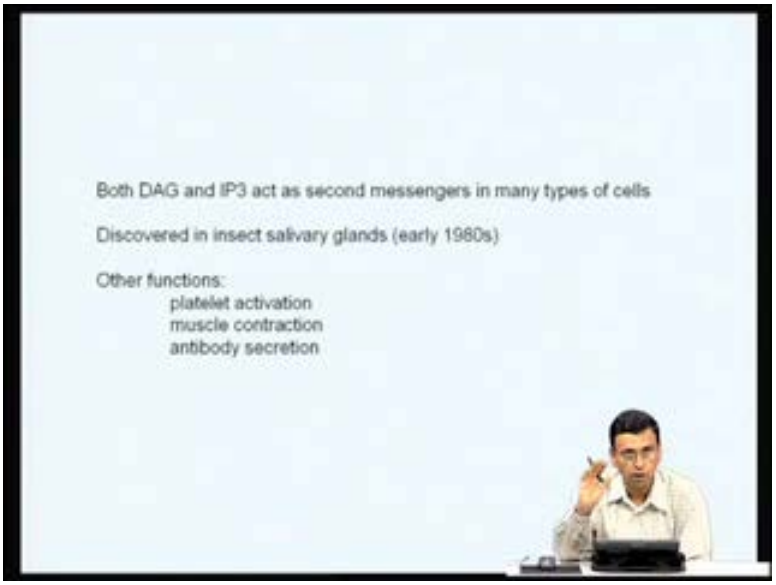

Both the diacylglycerol and inositol triphosphate, which have been produced by the action of phospholipase C and phosphatidylinositol bisphosphate, are very, very important second messengers, and they act on a number of… they perform number of physiological functions. These were first discovered in insect salivary glands in the early 1980s, and some of the important functions includes activation of platelets, muscle contraction, antibody secretion and so on and so forth, in addition to activation of gene expression programs.

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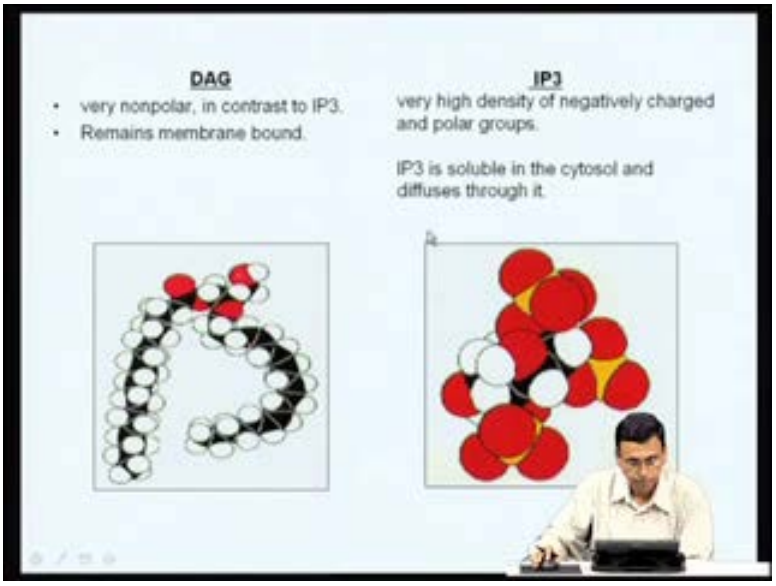

The diacylglycerol is a very non polar molecule. In contrast to IP 3, it is a very hydro– hydro– hydrophilic molecule. The DAG, therefore, remains membrane– membrane bound. You can see, the red actually indicates the polar groups; it is largely hydrophobic, and therefore, it remains associated with the hydrophobic membranes, whereas the IP 3 contains a lot of charged surfaces– number of polar groups– and therefore, it remains soluble in the cytoplasm and it can easily diffuse through the cytosol.

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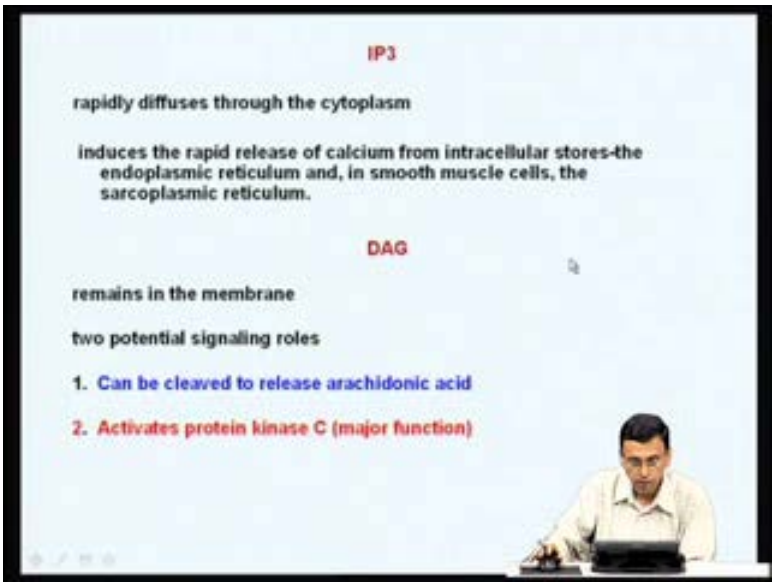

So, the inositol triphosphate, which has been synthesized by the action of phospholipase C on phophatidtylinositol bisphosphate, rapidly diffuses through the cytoplasm, and its primary function is to rapidly release calcium from intracellular stores– calcium stores– like the endoplasmic reticulum, or if it is smooth muscle cells, it is a called as sarcoplasmic reticulum. So, endoplasmic reticulum, which acts as a storage of calcium, the calcium is released from these store houses by action of inositol triphosphate.

On the other hand, the diacylglycerol, which is also the other molecule which has been synthesize by the…, by which, which is made by cleavage of phosphatidylinositol bisphosphate by phospholipase C, remains in the membrane. It has two very important roles– one is, it can be cleaved; it can be further converted to arachidonic acid, or it directly goes and activates protein kinase C along with calcium. So, our talk is properly going to focus up on how DAG activates protein kinase C, and what does protein kinase C activate, now, in the further downstream signaling pathways.

But I am just going to spend one minute just to introduce to you about the importance of arachidonic acid, which is another very important effector molecule that is synthesized from the diacylglycerol. So, do not be under the impression that diacylglycerol, primarily, is converted into– is involved in the activation of protein kinase C. Diacylglycerol is also converted into arachidonic acid, and as you can see, arachidonic acid is a very, very important molecule, and is the precursor for the synthesis of a number of molecules called eicosanoids.

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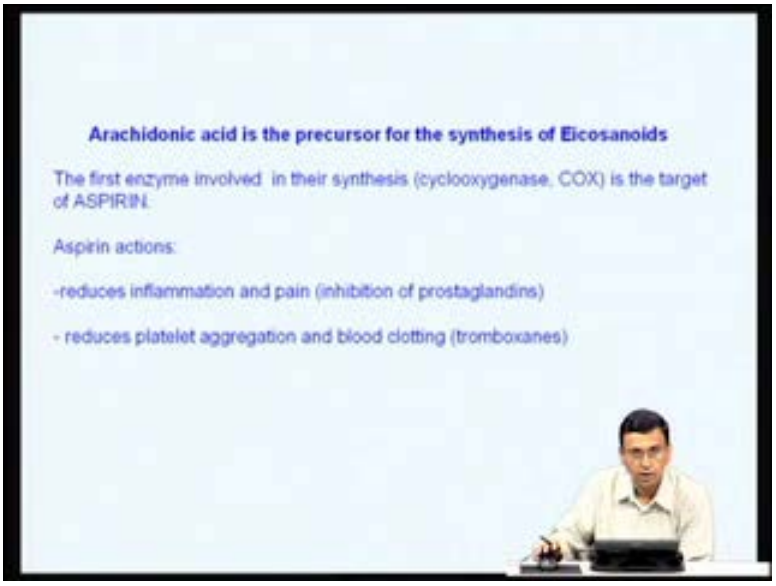

So, now, what are these eicosanoids? Eicosanoids are the molecules which are actually involved in the number of physiological processes, and the first enzyme involved in the synthesis, namely cyclooxygenase, is actually the target of aspirin. I am sure most of you have taken aspirin tablets whenever you would have pain or when you are a heart patient. When you actually want keep your blood thin, people usually recommend you take aspirin, and you can see the two important part produce of aspirin is that it reduces platelet aggregation, and therefore, prevents the blood clotting, and it reduces inflammation and pain.

And it does both these processes by actually inhibiting molecules called the enzyme called cyclooxygenase, and cyclooxygenase is the first enzyme which converts arachidonic acid into the eicosanoids– eicosanoids. So, diacylglycerol is not only involved in the activation of protein kinase C, but they also is the precursor for the synthesis of eicosanoids, which play a very important role in inflammation, pain, as well as platelet aggregation and blood clotting. So, just remember this, but we are not going to discuss this point in detail, but it is a very, very important pointer, (( )) because this particular pathway involving the synthesis of eicosanoids as well as the enzymes of this particular pathway is a billion dollar industry, and number of molecules that we are using today are all involved either activators or inhibitors of these particular enzymes involved in this particular pathway.

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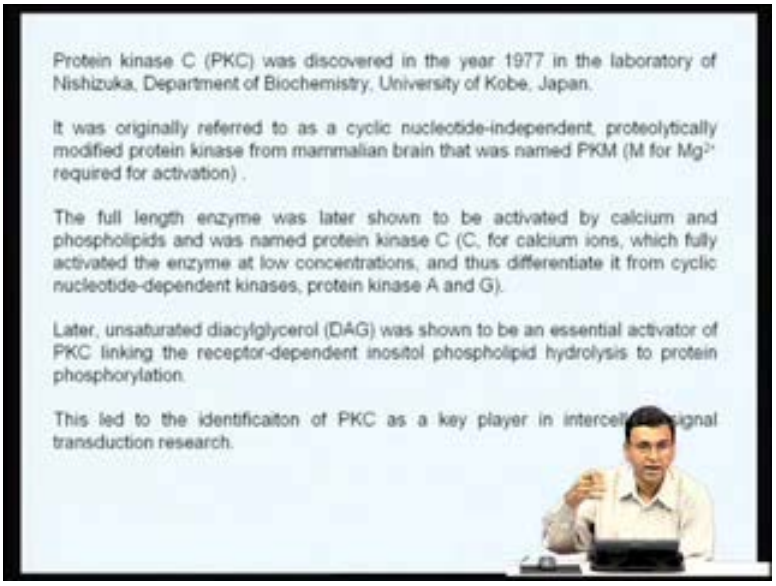

Now, let us come back to protein kinase C. So, once a diacylglycerol is synthesized by the action of phospholipase C, protein kinase C now has to go and then phosphorylate number of effector molecules. Let us now discuss very briefly about what is protein kinase C and how was discovered. Protein kinase C was actually discovered in the year 1977 in the laboratory of Nishizuka in the University of Kobe in Japan. So, the PKC was actually discovered in the year 1977; it was originally referred to as the cyclic nucleotide independent proteolytically modified protein kinase from mammalian brain, which was actually named as PKM, primarily because magnesium is required for its activation.

The full length enzyme was later shown to be activated by calcium and phospholipids, and was actually named protein kinase C– C stands standing for calcium ions, which is fully, which is required for activation of this enzyme at very low concentrations, and the enzyme was named as protein kinase C, primarily, to differentiate from the cyclic nucleotides dependent kinases, like protein kinase A and protein kinase G, which activate the cyclin dependent protein kinases, and the which refers the cyclic GMP dependent protein kinases.

So, like unsaturated diacylglycerol are shown to be essential activators of protein kinase C, and this particular discovery of linking DAG to the PKC actually signaled a very, very important role for PKC in the signal transaction pathway, and thus began a very intense investigation of the understanding the role of PKC– the signal transaction pathways– in

leading to the activation of a metabolic enzymes, ion channels, as well as transcription factors.

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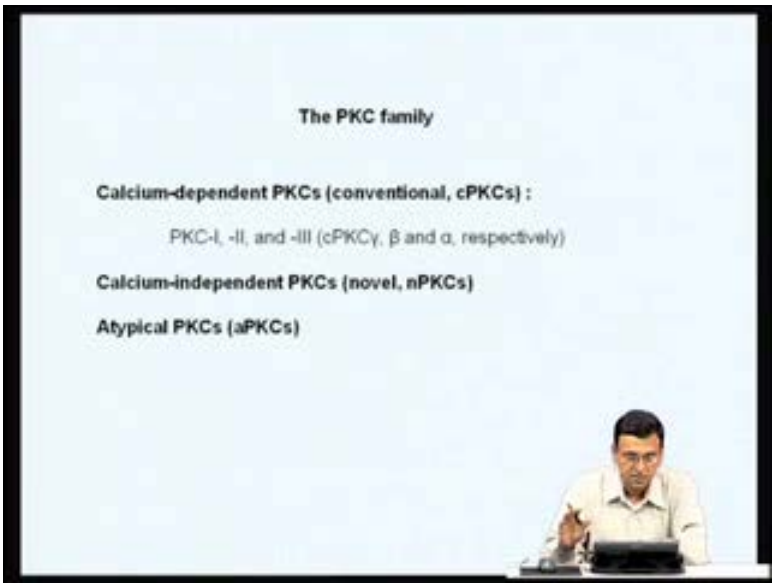

The protein kinase C family, ever since its discovery way back in 1977, consists of a number of members, and they are broadly categorized to three groups– the calcium dependent PKCs are the conventional PKCs, designated cPKCs. It contains of PKC 1, PKC 2, and PKC 3, also known as cPKC gamma beta and alpha. There is also calcium independent PKCs, which are actually novel PKCs or nPKCs, and also what are known as atypical PKCs or aPKCs, and in the next slide I have actually shown the various domain structures of all these various members of the PKC family.

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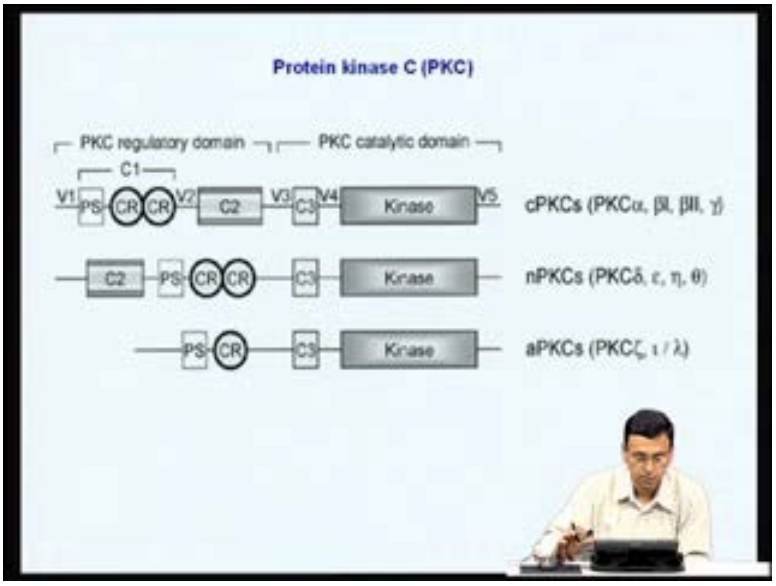

Basically, they contain what is called as a PKC regulatory domain and PKC catalytic domain, but we are not going into the details of the various structural details of the PKC family members. There are references, which I have listed at the end of this lecture series, and if you read some of these review articles, you will get more idea about the structure and function of these various protein kinase C members in this family, but our primary goal here is to understand the role in the regulation of gene expression.

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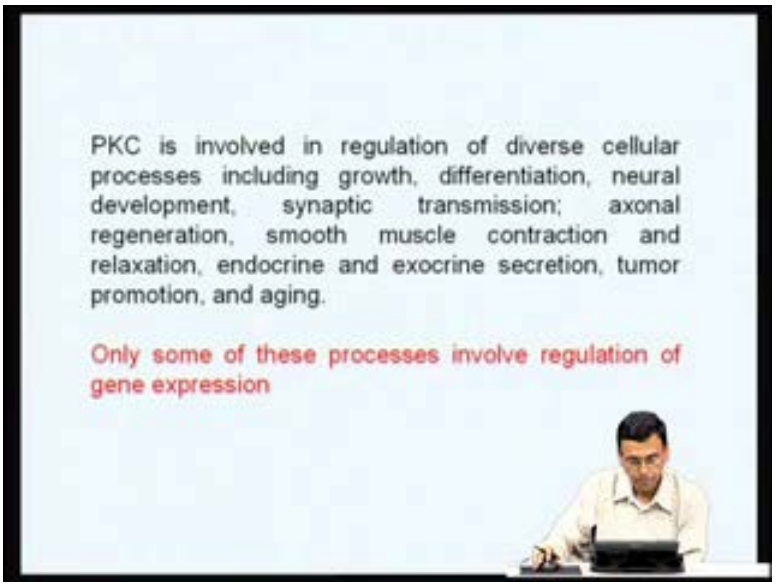

So, the protein kinase C is primarily involved in the regulation of diverse cellular processes, including growth, differentiation, neural development, synaptic transmission, axonal regeneration, smooth muscle contraction-relaxation, endocrine-exocrine secretion, tumor promotion, and aging. You can see, PKC plays a very important role in regulating a number of cellular processes, but remember, as I keep telling, many of these protein kinases, whether it is protein kinase C or calcium calmodulin kinases or protein kinase C, they all regulate this cellular process through different means, and regulation of gene expression is only one part of it.

So, they can directly go and activate certain metabolic enzymes or they can inhibit metabolic enzymes; they can go and influence certain the some structural proteins or cytosolic proteins or they may either influence on ion channels, but we are not going to discuss any of these aspects in this lecture series. We are going to focus, primarily, our attention on how these protein kinases involve or involved in the regulation of gene expression; what kind of transcription factors are activated or repressed by these protein kinases, and we will not discuss about the their effects on metabolic enzymes, cytosolic proteins, and ion channel proteins in this lecture series.

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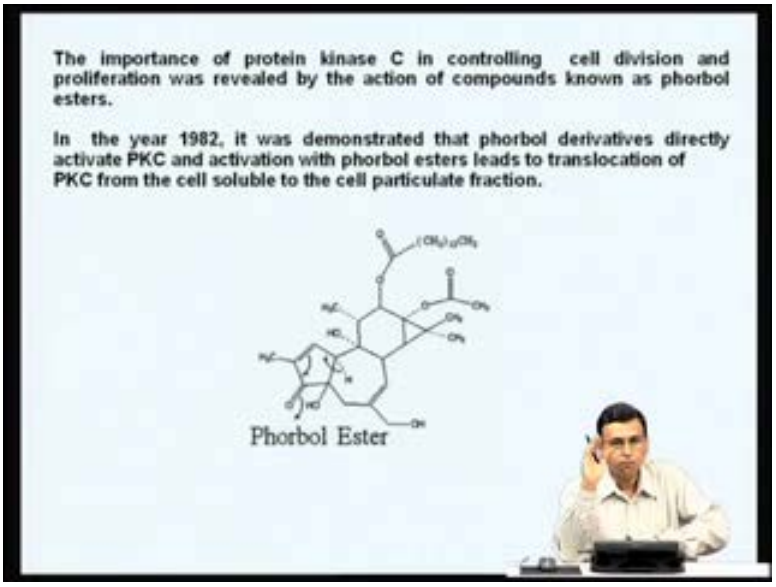

The importance of protein kinase C in controlling cell division and proliferation was revealed by the action of compounds known as phorbol esters. What are the phorobol esters? They, they are actually in the year 1982, the phorbol esters were shown to

directly activate protein kinase C, and activation of phorbol esters were shown to lead to translocation of protein kinase C from the cell soluble to the cell particulate form. So, this is the very, very important discovery in the field of protein kinase C research, and I have shown here the structure of phorbol esters, and let us see how exactly phrobol esters activates in the protein kinase C.

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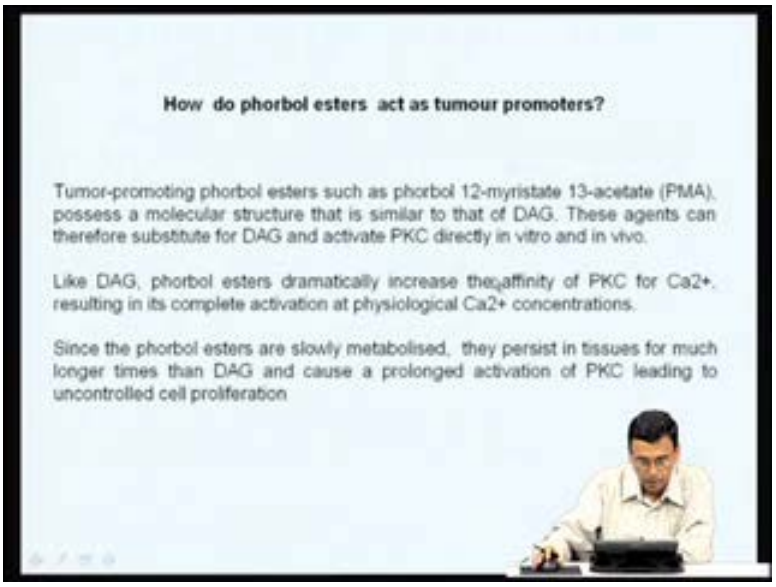

Now this tumor promoting phorbol esters such as phorbol 12 myristate 13 acetate known as– abbreviated as– PMA, they actually possess a molecular structure very similar to that of diacylglycerol and we know diacylglycerol and calcium regulate protein kinase C function; they are they activators of protein kinase C, and these PMAs are phorbol esters and have a structure which is similar to the diacylglycerol.

Now, these agents can, therefore, substitute for DAG and activate protein kinase C either directly in vitro or in vivo. So, if we treat the cells with this phorbol esters, they directly diffuse across the plasma membrane directly by protein kinase C and activate the protein kinase C signaling pathway, whereas the normal effector signaling molecules bind to their cognate receptors present on the cell surface, which in turn lead to the activation of specific G-proteins, and G-proteins activate phopholipase C, and phospholipase C then produces diacylglycerol and this diacylglycerol now activates protein kinase C.

So, if we treat cells with phorbol esters, it completely a bypasses this signaling mechanism, and this phorbol esters directly go and bind to protein kinase C and activate.

So, like diacylglycerol, phorbol esters dramatically increase the affinity of protein kinase C for calcium, resulting in its complete activation at physiological calcium concentrations. So, since phorbol esters are slowly metabolized, they persist in the tissues for much longer than DAG, and therefore, cause prolonged activation of PKC, leading to uncontrolled cell proliferation.

The PKC plays a very important role in regulation of a cell proliferation, and if PKC is continuously activated, it will result in uncontrolled cell proliferation. So, if you have molecules like phorbol esters, if you take cells with molecules like phorbol esters, since these phorbol esters go and directly bind to protein kinase and they are not degradable, very rapidly, it results in continuous activation of protein kinase C, and as a result, there is a continuous uncontrolled cell proliferation leading to cancer.

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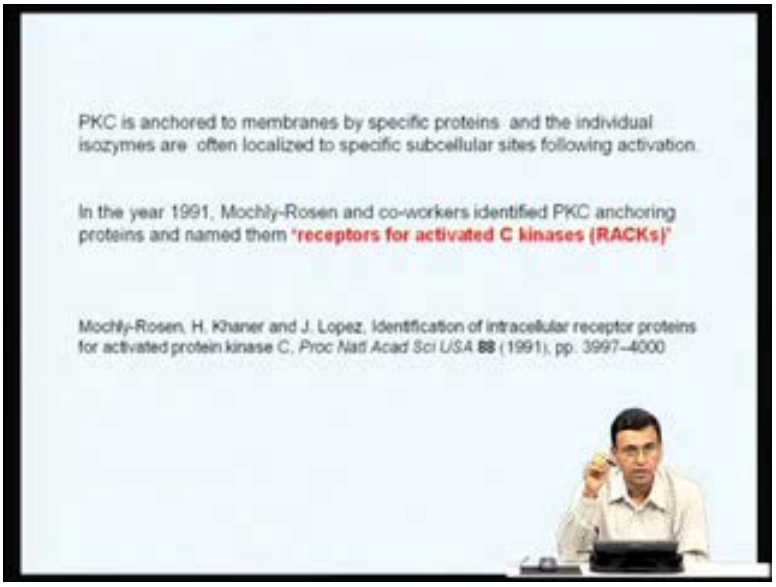

So, a very important aspect of protein kinase C research was the discovery that the protein kinase C is actually anchored to membranes by specific proteins, and the individual isozymes are often localized to specific sub-cellular sites following their activation. For example, in the year 1991, Mochly-Rosen and his co-workers actually identified PKC-anchoring proteins and named them as receptors for activated C kinase or RACKs.

So, there is another level of regulation as far as protein kinase C is concerned. Depending up on whether this protein kinase C is anchored to these RACKs or not, there could be a

regulation. So, the protein kinases may remain inaccessible for regulation in the anchored to protein, and when they are removed from their anchoring proteins then they become available to do the catalysis, and therefore, regulate various cellular possesses. So, depending upon the various tissue types, different kinds of RACKs actually holds these protein kinase molecules in a specific sub cellular location, and these constitute another important regulation of protein kinases signaling. There is a very nice paper that actually discovered (( )) the RACKs in detail the PNAS paper in 1991.

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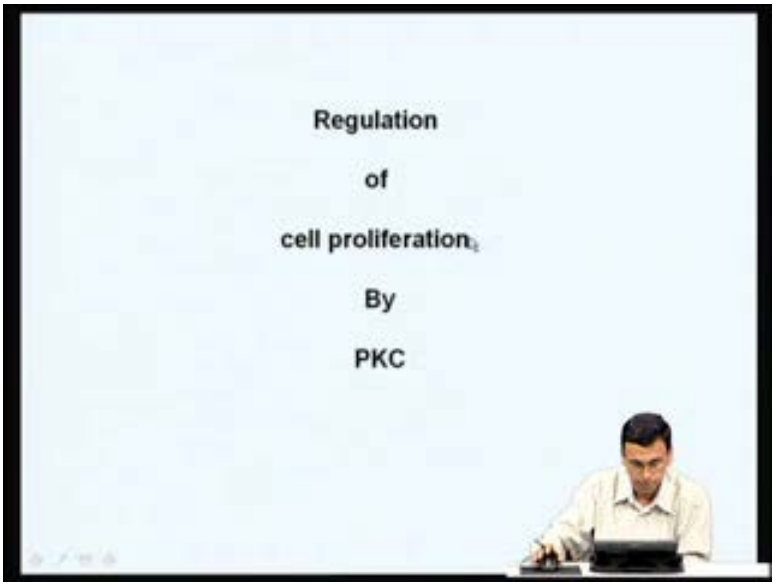

So, let us now briefly discuss about how protein kinase C regulates cell proliferation. So, so far, we have discussed how molecules binding to the GPCRs ultimately result in the activation of trimeric G-proteins, and these trimeric G-proteins, which contain the G q alpha subunit, now activates phospholipase C, and phospholipase C, now the phophatidtylinositol bisphosphate or PIP 2 into diacylglycerol and inositol triphosphate. Inositol triphosphate, in turn, goes and then activates the calcium channels in the endoplasmic reticulum; it is also in a huge increase in intracellular calcium release, and this calcium along with the diacylglycerol now goes and binds to protein kinase C and then activates the protein kinase C. Now, once the protein kinase C is activated, what does it do?

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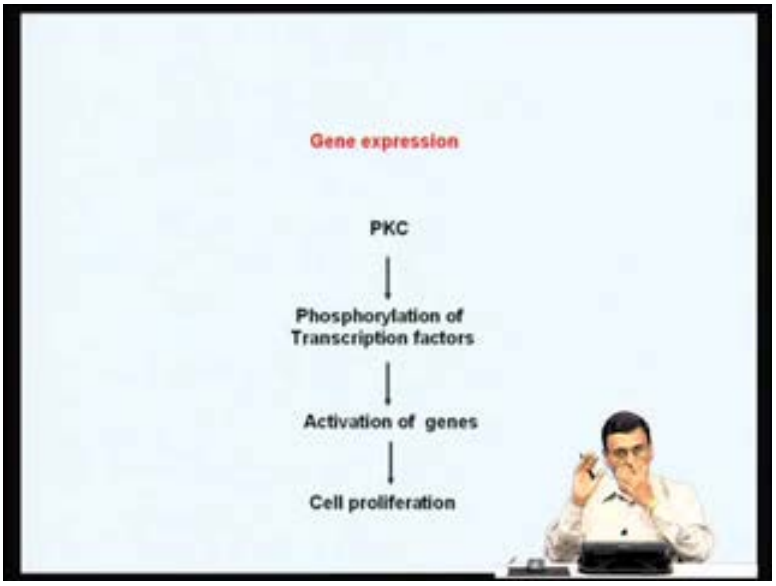

What are one of the main functions of protein kinase C? Especially the major mechanism by which protein kinase C activates cell proliferation path is, is actually by activating expression of very specific set of genes. So, the protein kinase C acts as a regulator of gene expression, primarily by phosphorylating some specific transcription factors, which on activates specific genes which are involved in the cell proliferation pathway, leading to cell division and cell proliferation. So, this is one of the important physiological processes regulated by protein kinase C.

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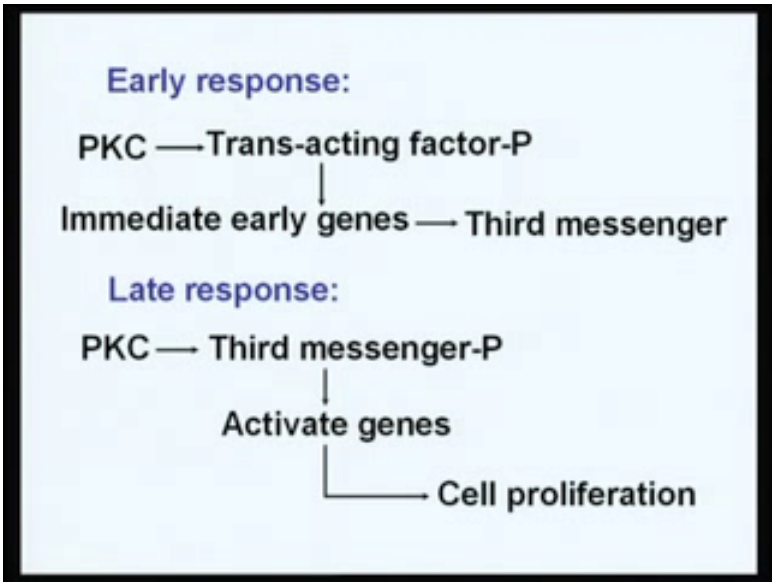

Now, it turns out, cell proliferation, in general, is happens in two different phases: one is called the early response, and another is called the delayed response or the late response. Now, very interestingly, the protein kinase C plays a very important role, both in the early response as well as in the late response. Now, let us see– this is a brief schematic sketch that actually tells you how protein kinase C acts as a regulator of cell proliferation; turns out, the moment protein kinase is activated by diacylglycerol and calcium, protein kinase C now goes on and phosphorylates specific transcription factors, which turns on the expression of a set of genes known as immediate early genes.

Now, these immediate early genes are so named because their expression is induced within minutes after this growth promoting stimulus– within 15 minutes or 20 minutes after you add a particular stimulus, which is known to be, you know, a cell proliferation, this set of genes are activated.

These immediate early gene products are often referred to as third messengers; they, in turn, again are phosphorylated by…. They get translated; that is when, when the immediate early genes are transcribed, they are translated into specific proteins. Usually, these proteins, again, have transcription factors, which are again phosphorylated by protein kinase C, and therefore, activated, they, in turn, go and activate all the genes involved in the cell proliferation. So, activation of immediate early genes as well as activation of genes involved in late response by protein kinase C, actually, marks the mechanism by which protein kinase C promotes cell proliferation. Let us discuss in detail how exactly these cell proliferation pathways operate.

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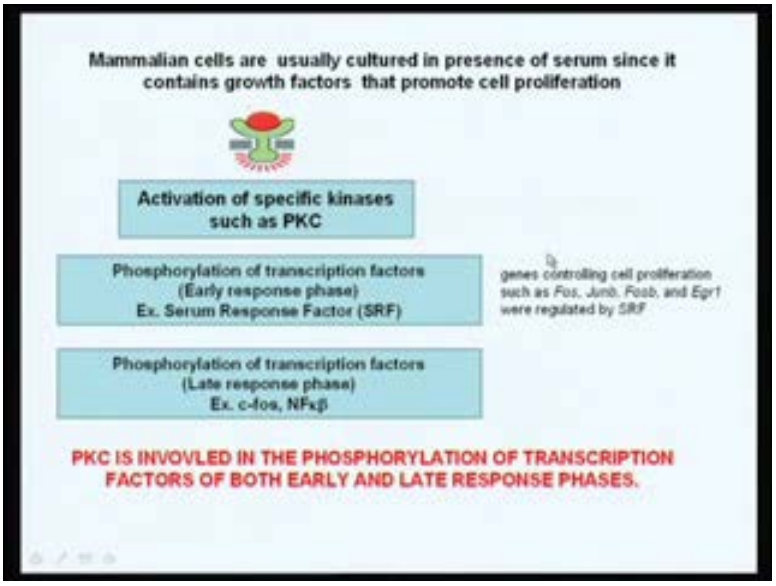

Let see for a taken example: I am sure many of you are familiar the way mammalian cells are cultured in vitro– in laboratory, in CO 2 incubators, usually, have petri plates where you take these mammalian cells like (( )) fibroblasts, or it could be primary cells or it could be transformed cells, and usually, whenever you want to culture these cells or cell lines in this– in laboratory– you do it in the presence of specific media, which contains the carbon source like glucose and various other ions, and so on and so forth.

But, it also contains a very important molecule called serum. Usually, either I have a fetal bovine serum or fetal calf serum– about 5 to 10 percent, and usually, mammalian cells are cultured in presence of this serum. Now, what does serum do? Serum is actually essential for the cells to proliferate; it turns out, the serum contains a number of growth factors, and when this growth factor binds to the specific receptors, it results in the activation of specific kinases, including the protein kinase C, and this, in turn, activates the immediate early response gene as well as late response genes, and that is how the cell proliferation program is initiated.

For example, as I mentioned earlier, the early response phase, we as soon as protein kinase C in activated, it first activates a set of early response phase, which actually involves phosphorylation of proteins called as serum response factor or SRF. The SRF is a very important transcription factor, which has binding element in the promoters of immediate early genes such as c-fos, jun-b, fos-b, egr-1, and so on and so forth.

So, all these genes known as the immediate early genes in their promoter element in their promoter contain response element for this serum response factor, and as a result, as soon as the serum response factor is phosphorylated by protein kinase C, the phosphorylated SRF now goes and binds serum response elements of these genes and activate the transcription of these genes, and as the result, all these proteins are actually made and all these proteins like Fos, Jun, and Egrf– they are all transcription factors– and once these transcription factors are made, they are again phosphorylated by protein kinase C, and therefore, activated, which in turn go and then activate target genes that are involved in various cell proliferation programs. So, this is how protein kinase C primarily activates cell proliferation pathways.

So, as I said– mentioned earlier– protein kinase C is involved in the phosphorylation of transcription factors of not only the early response phase, but also the late response phase; that means, it not only activates or phosphorylates transcription factor like serum response factor, but they also it also acts on proteins like C-Fos and NF kappa B, which are actually synthesized following the activation by SRF.

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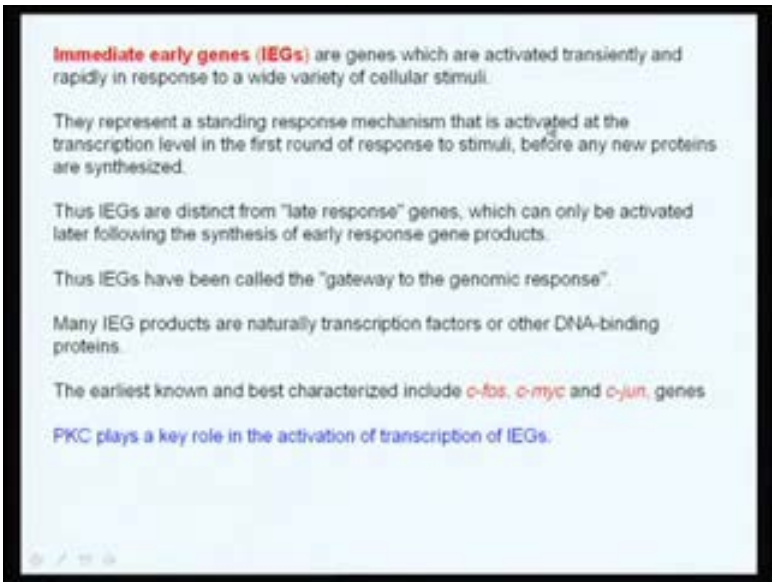

So, the immediate early genes are the genes which are activated transiently and rapidly in response to a wide variety of cellular stimuli– growth proliferating stimuli. They represent a standing response mechanism that is activated at the transcription level in the

first round of response to stimuli before any new proteins are synthesized. So, you can see, the early immediate early gene activation actually requires no protein synthesis.

There are already proteins like serum response factor in the…, in the cytoplasm, and they are phosphorylated by protein kinase C and that now initiates– triggers– transcription of immediate early genes. So, transcription of immediate early genes does not require any protein synthesis; it basically requires phosphorylation of preexisting transcription factors like SRF.

So, these immediate early genes are distinct from late response genes, which can only they activated later following the synthesis of early response gene products, like in the serum response factor now activates the expression genes like c-fos, cgr-1, and c-jun. They, in turn, activate the target genes, which are actually involved in the cell proliferation. So, the second (( )) that are activated by the immediate early gene products are known as the late response genes. So, the immediate early genes have been called therefore, the gateway to the genomic response. So, because without that, cell proliferation are possible. So, many of the immediate early gene products are, therefore, naturally transcription factors or DNA binding proteins.

So, one of the major functions of protein kinase C is to phosphorylate transcription factors like SRF, which is a transcription factor, and therefore, goes and binds to the promoter of immediate early, early genes and activate their expression, and these immediate early genes products are also transcription factors, because they have to now go and actually regulate the various target genes, which are directly involved in promoting cell proliferation . So, now, the best characterized immediate early genes are c-fos, c–myc, and c-jun, and they are all transcription factors and protein kinase C plays a key role in the activation of all these immediate early genes.

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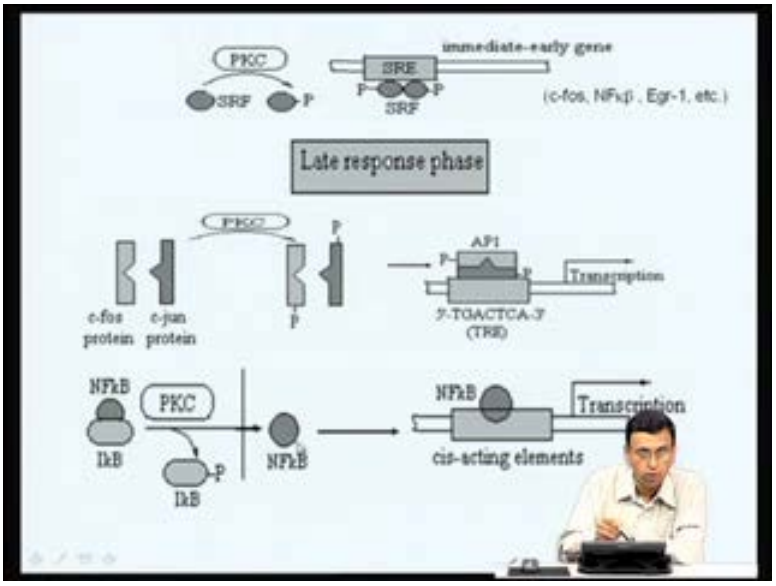

I have now given a very brief schematic of how the actually protein kinase C activates whatever I have discussed so far. Like I mentioned here, once the protein kinase is activated in the cell by enzymes like phospholipase C, and through the activation– through the binding of diacylglycerol and calcium, protein kinase C now phosphorylates the serum response factor, and the phosphorylated serum response factor now goes and binds to the serum response elements present in the promoters of immediate early genes, which could be c-fos, NF kappa B, egr-1 and so on and so forth, and as a result of this, all these immediate region of this transcribed; they are translated and the proteins like C-Fos, C-Jun, and NF kappa B are again phosphorylated the protein kinase C, and therefore, are activated.

And in the… For example, in the case of NF kappa B, the protein kinase C actually phosphorylates the inhibitor of the kappa B called the I kappa B, and therefore, the I kappa B can no longer interact with NF kappa B. Therefore, NF kappa B now becomes active, goes to the nucleus, and activates the target genes involved in cell proliferation, whereas in the case of the jun and fos, the both these proteins are directly phosphorylated by protein kinase C and in the the transaction, the phosphorylation actually takes place in the transcription activation domain, and as a result, they would not bind to the AP 1 response element of the growth genes involved in the cell proliferation, leading to their activation and leading to cell proliferation and cell division.

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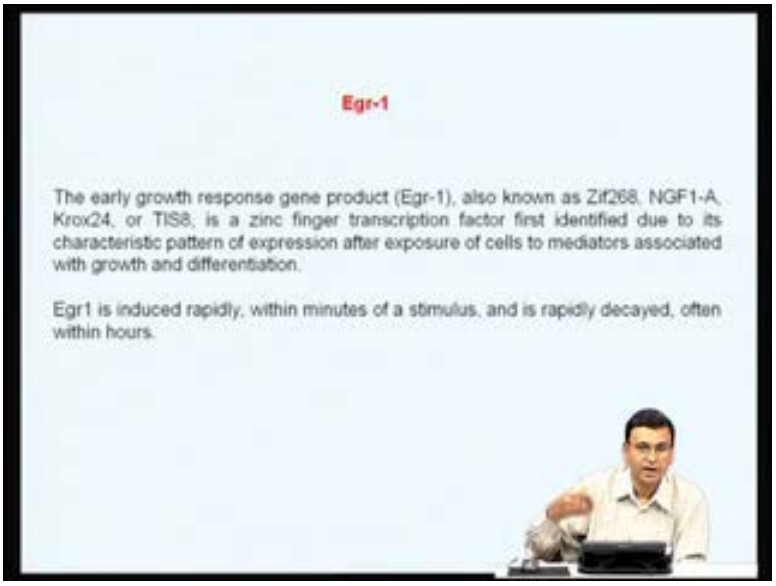

Among these proteins, the C-Fos, C-Jun are called as in the AP1; we have already studied in detail when we studied about the transcription factors, their various DNA domain, transcriptional activation domain in our earlier lectures. If you remember very well, the AP1 or C-Fos, C-Jun are the are the transcription factors which contain the basic region and leucine zipper, through which they go and bind to a sequence called TGACTCA.

And I also mentioned in my lecture on the cyclic AMP response: this sequence of the AP1 response element TGACTCA is very, very slightly different from the cyclic AMP response element of the CREB binding side, which is TGACGTCA, whereas AP1 binds to TGACTCA– G is missing. Now, the other very important at immediate early response gene is a gene that is codes for transcription factor called Egr-1. It is (( )) because it actually means the early growth response gene product or Egr-1– it is also known as Zif268, NGF 1-A, Krox24, or TIS8. It is basically a zinc finger transcription factor, and has been shown to be immediately induced when cells are exposed to stimuli that promote growth and cell differentiation.

Egr-1 is induced very rapidly within minutes of a stimuli, and it is also rapidly decayed within hours. So, many of these immediate early response gene products, their levels are very finely controlled in such cells, and if these levels are tampered with or they are over expressed, it can actually lead to cancer. So, the levels as well as the activity of the early

growth response genes has to be regulated a very fine manner in the normal cells, and the one thing that happens in cancer cells is that many of these transcription factors are over expressed, and as a result, they continuously promote cell proliferation.

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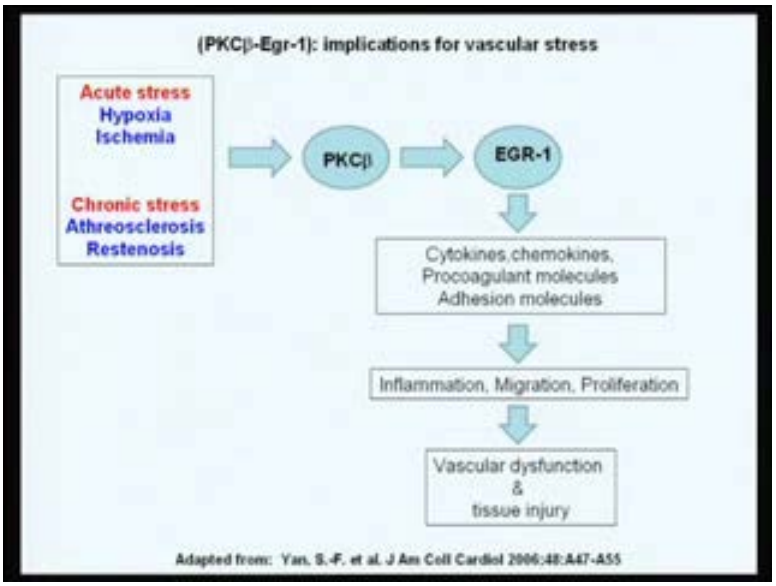

Now, I have given just one example, actually, taken from this particular article, just to give you an idea how the protein kinase C signaling is linked to the activation of Egr-1 expression. For example, in the case of (( )) means blood vessels, the Egr-1 signaling has been shown to play a very important role in vascular stress, like acute stress, hypoxia, ischemia, as well as chronic stress like athreosclerosis, restenosis. All these stress signals activate the PKC beta 1 of the members of PKC family of kinases, and the PKC beta activation results in the activation of Egr-1.

Which then results in the synthesis of various cytokines, chemokines, procoagulant molecules, adhesion molecules, and so on and so forth, leading to a number of pathological processes like inflammation, migration, as well as proliferation, leading to vascular dysfunction and tissue injuries. So, activation of PKC signaling pathway through the Egr-1 pathway plays a very, very impotent role in vascular stress and managing a number of vascular diseases.

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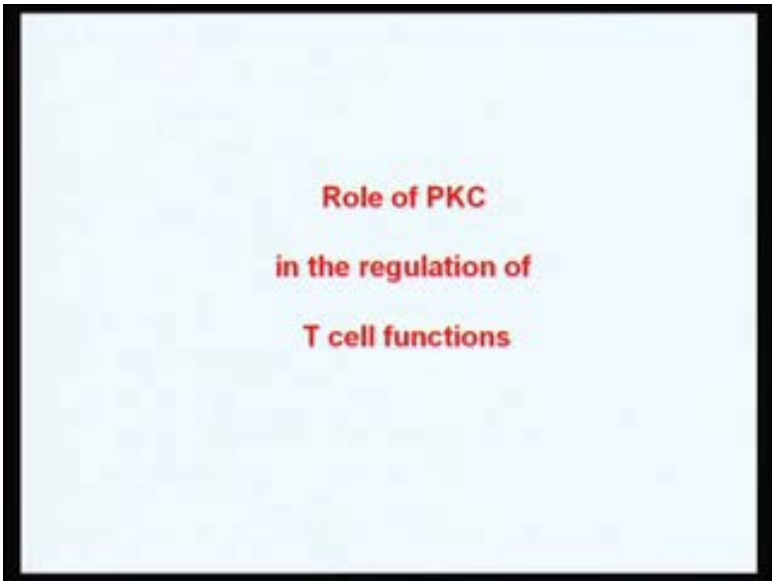

Now, let us try to spend some time to understand what is the role of PKC in regulation of T-cell functions. So, so far, I gave you one of the important pathways which are activated by PKCs in cell proliferation pathways involving immediate early response gene, which like Egr and C-Fos, which in turn go and activate the actual target genes involved in cell proliferation. Now, let us try to understand another example of how protein kinase C is actually involved in the regulation of various T-cell functions.

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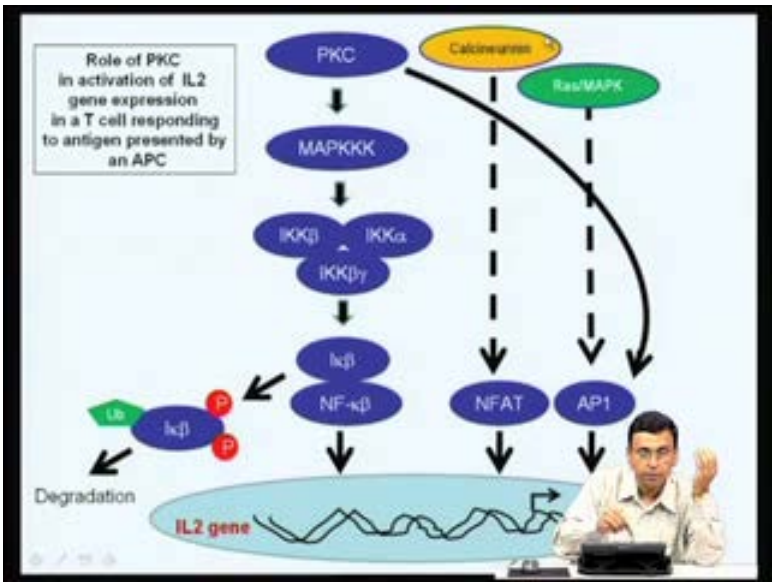

Now, here is a cartoon that, actually, I have drawn, to basically tell you how the protein kinase C plays a very, very important role in the synthesis of cytokines like interleukin 2 following the binding of antigen presenting cell to the T-cell receptors. The APCs containing the antigen bound to the MHC molecules, when it docks with the T-cell, it results in the activation of the T-cells, and the results in the production of interleukin 2, and this cartoon basically shows how protein kinase C plays a very, very important role in the activation of IL2 gene expression when the antigen or when the antigen presenting docks with the T-cell receptor.

The docking of the antigen presenting cell with the T-cell receptor activates the number of signaling pathways; it is called T-cell receptor signaling pathways. We are not going to all the details, because in order to understand all the T-cell signaling, we also need to understand other signaling mechanisms, which we are going to discuss in the end.

Suffice to know at this stage that one of the signaling pathway, which are activated by the docking of the antigen presenting cell or binding of the antigen presenting cell MHC molecule with the T-cell receptor is the activation of… leads to activation of protein kinase C, and this protein kinase C, through the MAP kinase pathway, now phosphorylates the inhibitor of kappa B or the I kappa B, and once the inhibitor of the kappa B is phosphorylated, it is ubiquitinated and it is degraded, and therefore, the NF kappa B, which is actually held in inactive form in the cytoplasm, now becomes activated, and that enough kappa B now goes and binds to the promoter of IL 2 g and activates this expression.

So, now, for the efficient activation of IL 2 gene, the IL 2 gene has to be… be activated not only the NF kappa B, but also by other transcription factors like NFAT, AP1, and so on and so forth. So, the promoter of IL2 gene contains binding sites for all these three transcription factors, like AP1, NFAT, and NF kappa B, and one of the mechanisms by which NF kappa B is activated is through the protein kinase C signaling pathways, but this NF kappa B plays a very, very important, very complicated transcription factor, which is activated by multiple signaling pathways.

The same way, similarly, for example, for the efficient activation of the IL2 gene, not only you need NF kappa B, you also require other transcription factors NF2AT and AP1, which are activated by other pathways. Now, the AP1 is activated by the ras map kinase

pathway, but the AP1 also directly phosphorylates protein kinase C, and therefore, it can be activated directly whereas, NFAT is actually activated by a, a calcium dependent pathway, which I have discussed in the next slide, which results in the activation of calcineurin– it is a phosphatase, which actually dephosphorylates NFAT, and NFAT is inactive in the phosphorylated form– is active in the dephosporylated form– and one FAT is dephosporlated by the calcineurin, NFAT now goes into the nucleus, bind into the response element, and activates transcription.

So, three important transcription factors, which are required for IL2 gene expression, namely, the NF kappa B, NFAT, and AP1 are activated by multiple signaling pathways during the T-cell receptor activation by the antigen presenting cell, and of which the NF kappa B activation directly involves PKC mediated map kinase pathway activation, and PKC can also directly phosphorylate AP1, as NFAT is activated rather indirectly, whereas the calcium, which is actually released by the by the action by the action of phosphorylate IPC that the the calcium that is released by the inositol triphosphate, which is produced by phospholipase C activation. The calcium then activates the calcineurin; then calcineurin, in turn, dephosphorylates NFAT, and NFAT then goes and binds go to the nucleus– binds and activates the transcription of the gene. I want to just spend one important, maybe one minute on this calcineurin pathway, because it has a very important role in certain biomedical applications.

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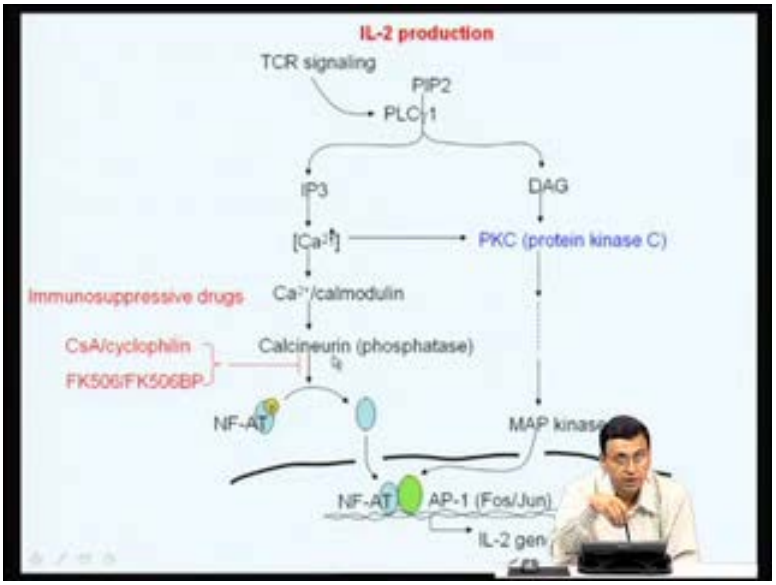

This is again a cartoon, which actually a very simplified version what I have just told you. During T-cell receptor signaling, the phosphatidylinositol bisphosphate is cleaved by phospholipase C into inositol triphosphate and diacylglycerol. Diacylglycerol now activates protein kinase C; protein kinase C, through the map kinase signaling, now activates the AP1 as well as NF kappa B. Now, the inositol triphophate that is also produced by the phospholipase C action or cleavage of phosphatidyl inositol bisphosphate, now releases the intracellular calcium levels.

This calcium now binds to calmodulin, and the calcium calmodulin activates calcineurin, which is actually phophatase. Calcineurin now dephosphorylates NFAT, and dephosphorylated form of NFAT now goes into the nucleus, bind to the response element; together, with AP1 as well as NF kappa B, now activates– induces– IL2 gene expression.

Now, a very important aspect about this: I want to spend some time to just to emphasize the fact that understanding these signaling pathways and understanding the various transcription factors, which are activated or repressed by the activation of these signaling pathways is very, very important for you to develop novel drug molecules, because many of these components of the signaling transduction pathways either at the transcription factor level or at the kinase or phosphatase level, there very important drug targets.

Here is one example: the calcineurin, as I told you, is very, very essential for the activation of NFAT, and a very important immunosuppressive drug like cyclosporine actually act as a inhibitors of calcineurin, and as you can see in the next slide, cyclosporine is very immunosuppressive.

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Those people who undergo something like kidney transplantation or organ transplantation, for example, they are usually treated with immunosuppressants, because you want to prevent rejection of the transplanted organs. So, one of the molecules, which are actually given for drugs which are given to these patients, are immunosuppressive like cyclosporine, and cyclosporine primarily acts by inhibiting the calcineurin.

Therefore, NFAT is not activated; therefore, IL2 is not sync– synchronized– and therefore, the… the antigens or the alloantigens, which are present in tissues, are not recognized by the immune system, and therefore, there is immunosuppression. So, a major consequence is the inhibition of the activation of the transcription factor NFAT, thus preventing the rejection of foreign organs or bone marrow, or even kidney transplantation, by disrupting the signaling pathway that activates T-cells.

So, we can see how understanding the signal transduction pathways and how understanding which transcription factors are activated by which signaling pathway is very, very important for you to develop drug targets and novel drug molecules, and by knowing that activation of the PKC signaling pathway results in the increase in the calcium levels, these calcium levels, in turn, through the calcium calmodulin pathway, results in the activation of calcineurin, and calcineurin now dephosphorylates NFAT, and therefore, activating NFAT, and that is very, very essential for IL2 gene expression.

And this knowledge has now led to the development of immunosuppressive molecule cyclosporine, which actually inhibit calcineurin and therefore, the IL2 expression can be prevented. So, understanding these signaling pathways and how transcription factors are activated by the signaling pathway is very, very essential for developing new drug molecules as well as for the identification of drug targets, and this is a billion dollar industry.

So, in order for you to understand– appreciate– gene regulation, you have to understand signaling molecules or signaling pathways. If you do not understand how cells communicate with extracellular signaling molecules, and how extracellular signaling molecules, through a various cascade a signaling pathways in affects a specific gene expression program, without this knowledge, the you we cannot appreciate how gene expression takes place, and how transcription factors or transcription or gene expression programs can be made use of for the benefit of mankind.

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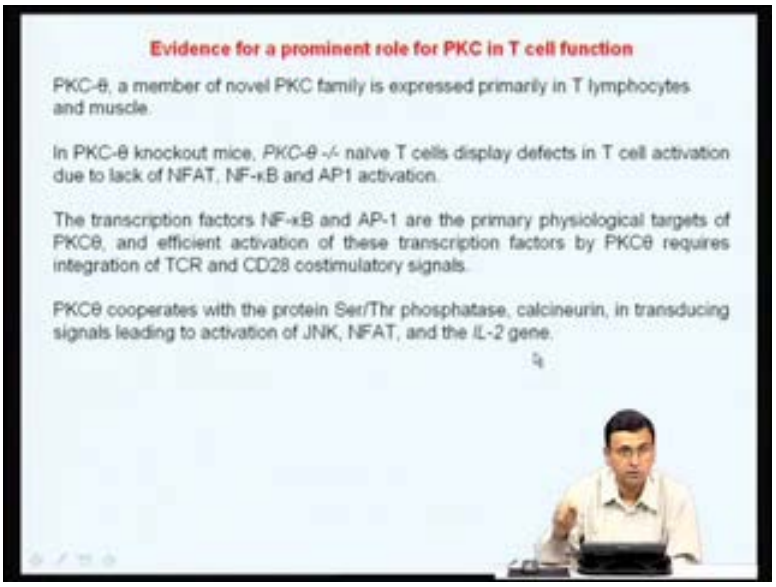

The protein kinase C what I have told you, so far, is that protein kinase is plays a very, very important role in the antigen presentation, leading to the or the activation of T-cell receptor following antigen presentation, and cytokines like IL2– IL2– are actually synthesized primarily by specific signaling pathways involving protein kinase C. Now, a number of evidences actually indicate that PKC plays a very, very important role in the not only in the activation of T-cell receptor, but also a number of T-cell signaling

pathways, and one best way of actually demonstrating this is to see what kind of protein kinase C actually expressed in T-cell.

Then, you knock out this particular PKC and see what is the effect of this knock out of PKC on the T-cell receptor function. In fact, such a things has been done. For example, one of the PKC isoforms, which are actually expressed in high levels in primary T-lymphocytes and muscles is called PKC theta, which is a member of the novel PKCs. I told you in the beginning there are three PKCs sub families and PKC theta is a novel PKC member of a novel PKC sub family of genes, and when you actually knockout this PKC theta, which is a major isoform of PKC which is expressed in T-cells, in these knockout T-cells the naive T-cells display defects in T-cell activation due to the lack of expression of NFAT, NF kappa B, and AP1, clearly indicating that protein kinase C plays a very, very important role in the activation of all these three transcription factors, and therefore, if you knockout PKC, you will not see the activation of these transcription factors.

And therefore, you do not see IL2 expression and transcription factors of NF kappa B and AP1 are primary physiological targets of PKC theta. I have already mentioned in the previous cartoon how the NF kappa B is activated by PKC pathway. NF kappa B is activated primarily by the phosphorylation of I kappa B, where the map kinase pathways involved in protein kinase C, and when I kappa B is phosphorylated and degraded by ubiquitin, NF kappa B is now goes in nucleus and binds to the NF kappa B– the response elements of the IL2 gene.

Similarly, AP1 is a directly target of protein kinase C, or it can also be activated by map kinase cascade. So, NF kappa B are direct targets for protein kinase C and the efficient activation of these transcription factors by PKC theta is required for the integration of T-cell receptor and CD28 co stimulatory signals. I am not… I am presenting a very, very simplified version of activation of T-cell receptors signaling involving PKC signaling, and it is much more complicated; it involves convergence of multiple signaling pathways, but idea is not to confuse the listener, but actually understand one aspect of T-cell receptor signaling.

And what I am trying to convey to you– the message– is that one aspect of T-cell receptor signaling involves protein kinase C, and protein kinase C is essential for the

expression of IL2– interleukin 2 expression, and for interleukin 2 expression, you require transcriptional factors like NF kappa B, AP1, and NFAT, and at least two of these transcription factors are activated by the PKC pathway and PKC theta cooperates with the protein serine threonine phosphatase calcineurin in transducing signals leading to activation of JNK, NFAT, and as well as the IL2 gene. So, protein kinase C plays a very, very important role in the T-cell function.

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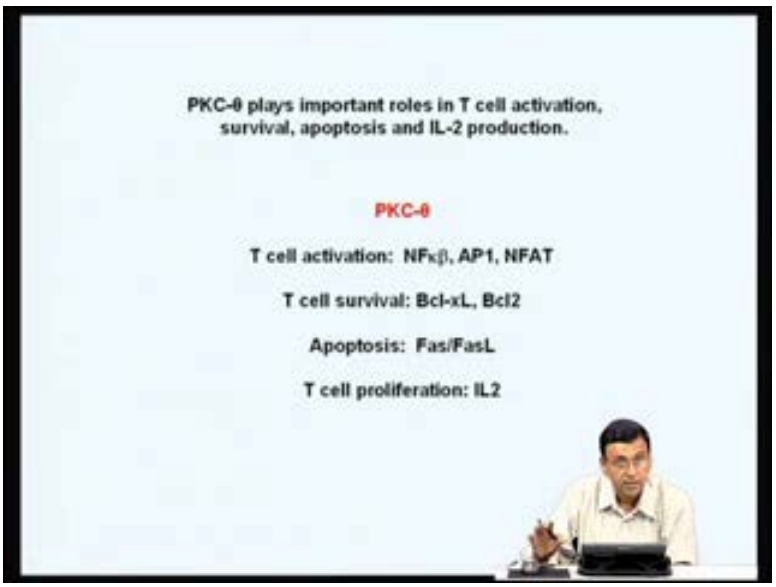

In fact, a number of studies actually shown that PKC theta plays a very important role not only in T-cell activation, but also in T-cell survival, apoptosis, as well as IL2 production. So, the as well as T-cell activation using this kind of PKC theta knockout mice, it has been very well shown that the protein kinase PKC theta plays a very important role in T-cell activation, because it is essential for the activation– for transcription– transcription factor like NF kappa B, AP1, NFAT; it is also plays a very important role in T-cell survival.

For example, it is involved in the activation of Bcl-xL and Bcl2 genes, which are involved in the apoptosis as a T-cell survival as well as in apoptosis. It regulates the expression of Fas and Fas ligand, and it is also involved in T-cell proliferation. As I just told you, how IL2 expression is directly regulated by the influence of transcription factors, so protein kinase C plays a very, very important role in number of T-cell function.

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What I would like now mention is to just take you a few minutes to tell you that there are…, there are umpteen number of examples that actually demonstrate how PKC plays a very important role and what kind of signaling pathways are activated by signaling– by protein kinase C– ultimately leading to the activation of specific transcription factors and specific target genes, and there are actually pathways which have been worked out.

And these pathways are actually present in number of journal websites, like for example, some of the signaling pathways are activated by protein kinase C are what are called myocyte adrenergic pathway, granule cell survival pathway, differential pathway, differentiation pathway involving PC12 cells. So, I am going to show some of the cartoons taken from the journal websites, just to tell you how PKC plays a very important role in these various signaling pathways.

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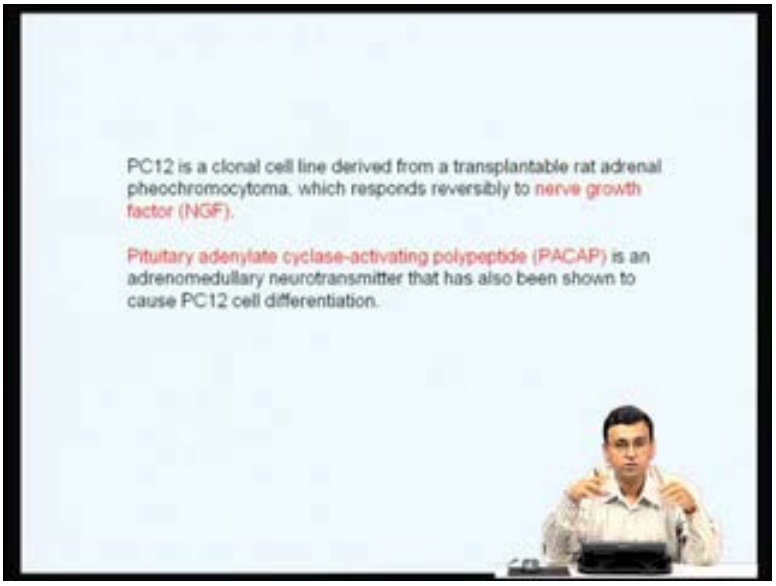

For example, PC12 is actually pheochromocytoma cell line, which is the clonal cell line derived from a neuronal cell line, and this is derived from transplantable rat adrenal pheochromocytoma cells, which responds reversibly to nerve growth factor. So, if we take PC12 cells and treat them with nerve growth factor, they differentiate into neurons. Now, using this model system, people have actually demonstrated what are the… what are the signaling pathways involved in the this differentiation program; how do these cells differentiate neurons, and it turns out, protein kinase C plays a very, very important role in this.

Nerve growth factor immediately differentiate PC12 cells into neurologic cells. In addition to nerve growth factor, another important effector molecule called pituitary adenylate cyclase activating polypeptide– PACAP– is also an adrenomedullary neurotransmitter that has also been shown to cause PC12 differentiation.

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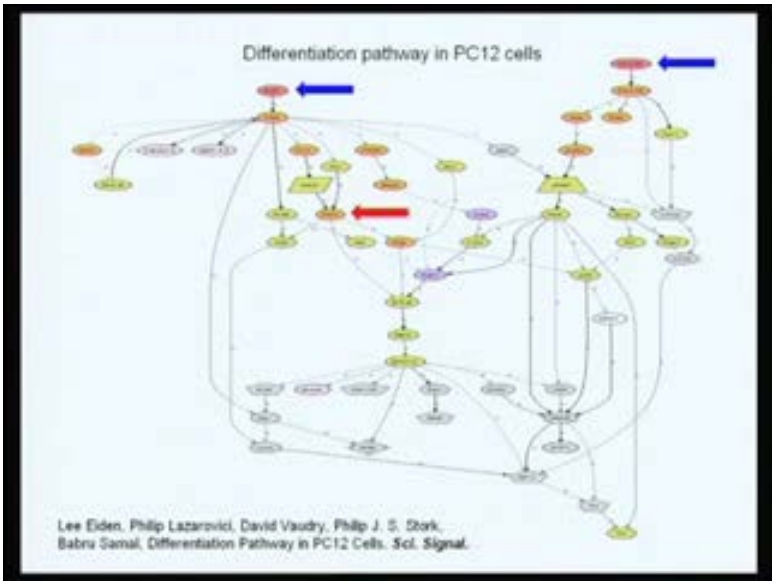

Now, this mechanism by which both these NGF as well as PACAP activate this program– differentiation program– of PC12 cell are very well worked out, and has been summarized in this particular cartoon taken from the Science Signaling website and you can see, nerve growth factor and the PACAP, as shown in the blue arrows here, activate two distinct signaling transaction pathways which converge and all, all like the NGF pathway actually involves the activation of the protein kinase C here, which through again the map kinase pathway, activates, ultimately, the transcription of c-jun, Egr-1 and so on and so forth, or AP1 and both these factors through two distinct signaling pathways– one involving the G-protein signaling couple pathway as well as the CREB pathway can activate the differentiation program and the nerve, nerve growth factor activates through the PKC pathway.

So, using these kinds of model system, people have actually drawn these signaling networks and such signal transaction pathways are brought out for a number of physiological processes, and I am just going to show two more examples to actually show PKC involvement in such signaling pathways. So, PKC plays a very, very important role in the differentiation of PC12 into neurons.

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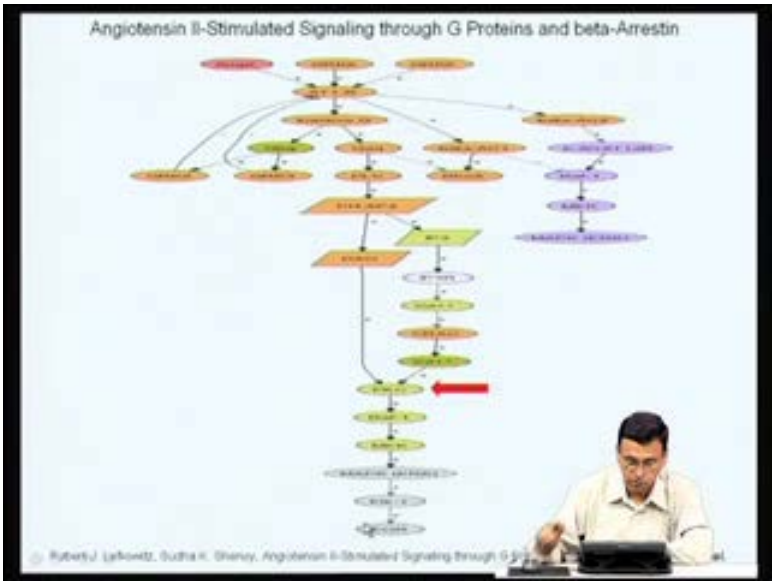

Again, the angiotensin-2 stimulated signaling through G-proteins and beta arrestins again involves the PKC pathway. I do not expect you to go through all the details here, but just you can see, when these molecules bind to the receptors, it results in the activation of phospholipase C, results in synthesis of inositol triphosphate and diacylglycerol, and inositol triphosphate results in the calcium release, and both the DAG and calcium activates the PKC, leading to the activation of downstream signaling molecules, ultimately leading to the activation of the early growth response.

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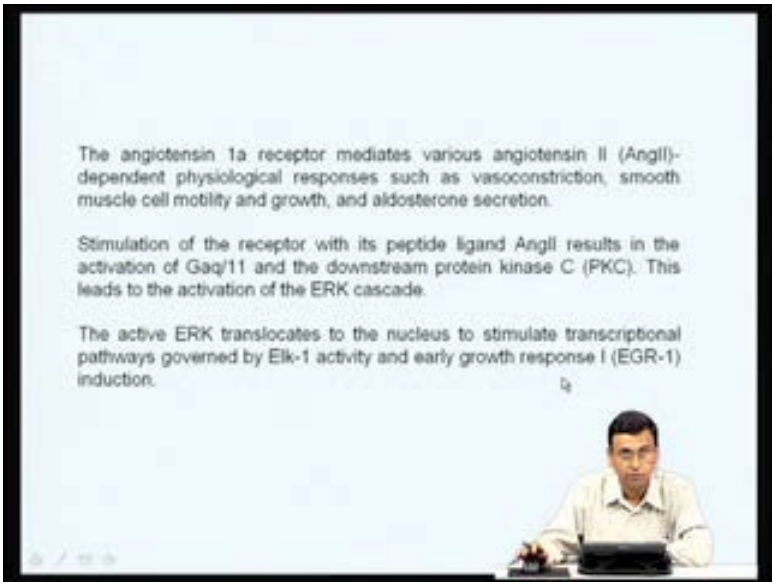

This is just the details of what exactly is angiotensin-1 receptor, I am not going into the details. Suffice to know that stimulation of the receptors with its peptide ligand angiotensin-2 results in the activation of Gaq-11subunit of G-protein– trimeric G-proteins– leading to the activation of protein kinase C. This due to the activation of the ERK or extracellular receptor kinase cascade. This extracellular receptor kinase translocates to the nucleus and stimulates transcriptional pathways governed by the Elk activity and the EGR-1 transcription factors, which is the early, early growth response gene product.

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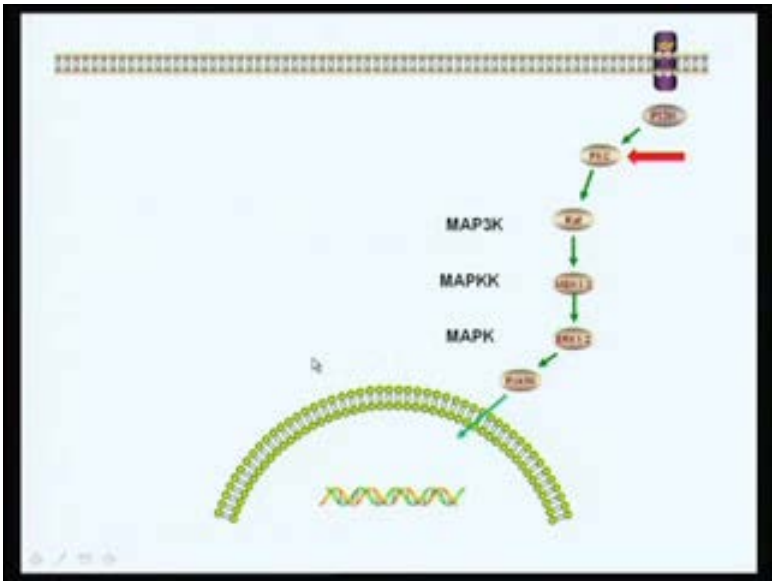

Similarly, insulin-like growth factor– again a very important molecule involved in cell proliferation– again acts through a PI 3 kinase pathway. This is a receptor tyrosine kinase pathway. We are going to discuss in the next class how– what– are these receptor tyrosine kinases. So, again, the insulin growth factor binds to its receptor; it results in activation of tyrosine kinase factor, and through the P I 3 signaling pathway, again, PKC is activated.

So, PKC is not only activated by the trimeric G-proteins; PKC can also be activated by the receptor tyrosine kinase pathways, and the activation of protein kinase C again results in the activation of map kinases. The three map kinases are shown here– the map three kinase, map kinase kinase, and map kinase, ultimately leading to phosphorylation of transcription factors and activating specific gene expression programs.

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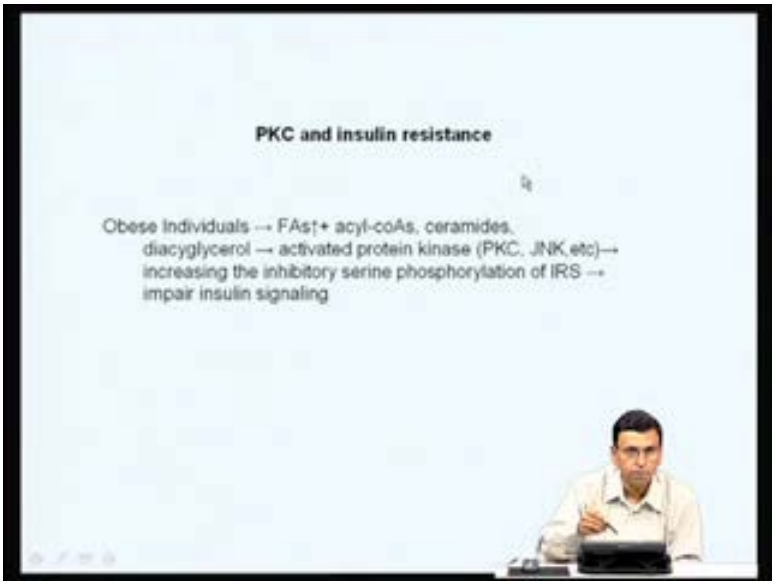

So, it is not possible to discuss all the signal transaction pathways. I have just given you few examples to just tell you how PKC plays very important role in activation specific gene expression programs. Now PKC is of extreme extraordinary biomedical importance because a number of disease processes are attributed to the improper activation or improper regulation of protein kinase C. For example, PKC has shown to be involved into the insulin resistance and diabetes; for example, in obese individuals fatty acids and acyl-CoAs, ceramides, they increase, and the diacylglycerol, as a result, all these molecules like fatty acid acyl-CoAs and ceramides– they activate the PKC signaling pathway through the diacylglycerol activation.

As a result, the serine phosphorylation of IRS, which is very important signaling molecule in the insulin receptor signaling pathway, results in impaired insulin signaling leading to diseases like diabetes. So, PKC plays a very important role in insulin resistance.

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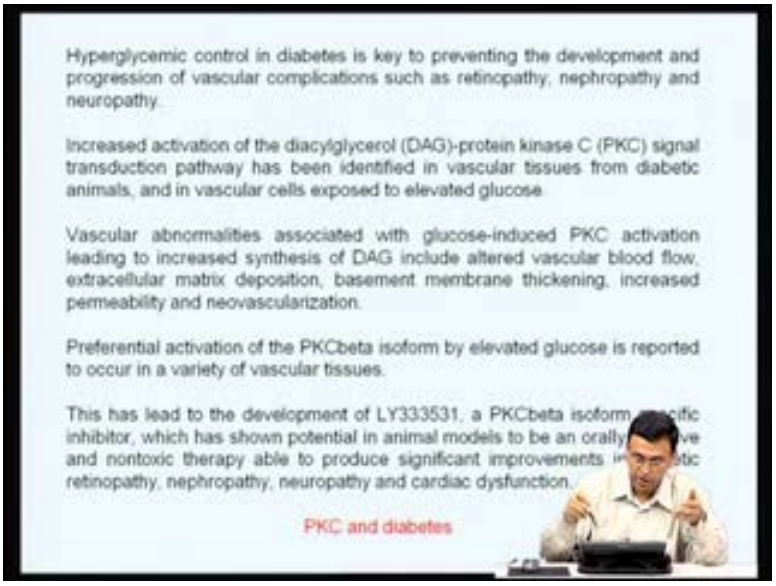

Similarly, PKC plays a very important role in diabetes. Hyperglycemic control in diabetes is a key to preventing the development and progression of vascular complications like retinopathy, nephropathy, and neuropathy may advance stages of diabetes patients. The one of the major complication diabetes patients is retinopathy, nephropathy, and neuropathy. These are all very, very complicated manifestations of diabetes, and it has been shown in all these ailments, increased activation of the diacylglycerol protein kinase C pathway has been identified as a major cause for the vascular tissue damage from the diabetic animals, and those vascular cells are exposed to elevated glucose levels. So, if you have high levels of glucose in your blood, it results in the activation of diacylglycerol protein kinase C pathway, resulting in all these kind of disease like retinopathy, nephropathy, and neuropathy.

Vascular abnormalities associated with glucose induced PKC activation leading to increased in the sub DAG includes altered vascular blood flow, extracellular matrix deposition, basement membrane thickening, increased permeability and neovascularization– these are all various clinical manifestations of advanced stages of diabetes. So, preferential activation of the PKC beta isoform by elevated glucose is reported to occur in a variety of vascular tissues of diabetes patients. So, what is the… what is the significance of this knowledge? People are actually trying to see whether these kinds of patients can be treated with some of the inhibitors of protein kinases. For example, a molecule like LY333531– a protein inhibitor of PKC beta isoform– has been

shown to be have a potential to be an orally effective and nontoxic therapy for producing significant improvements in diabetic retinopathy, nephropathy, and neuropathy, as well as cardiac dysfunction. So, inhibitors of protein kinase C have tremendous potential in treatment of disease like diabetes, especially some of the problems involved in diabetes.

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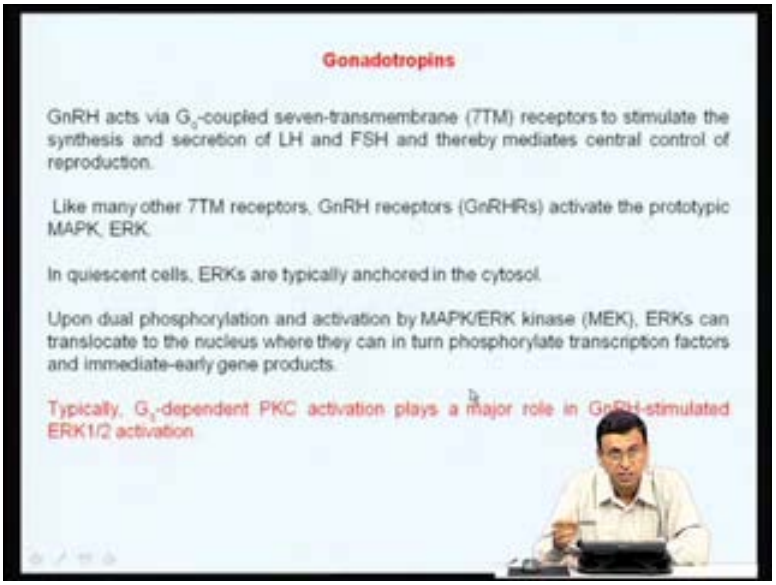

Similarly, gonadotropin hormones– FSH, LH– they are all activated through these G-protein coupled receptors– the seven transmembrane proteins. Again the G-protein dependent protein kinases C activation plays a major role in the GnRH stimulated extracellular receptor kinase pathway of the making of physiological responses. I will not go in to the details, just to tell you that the gonadotropin action also involves the PKC signaling pathway.

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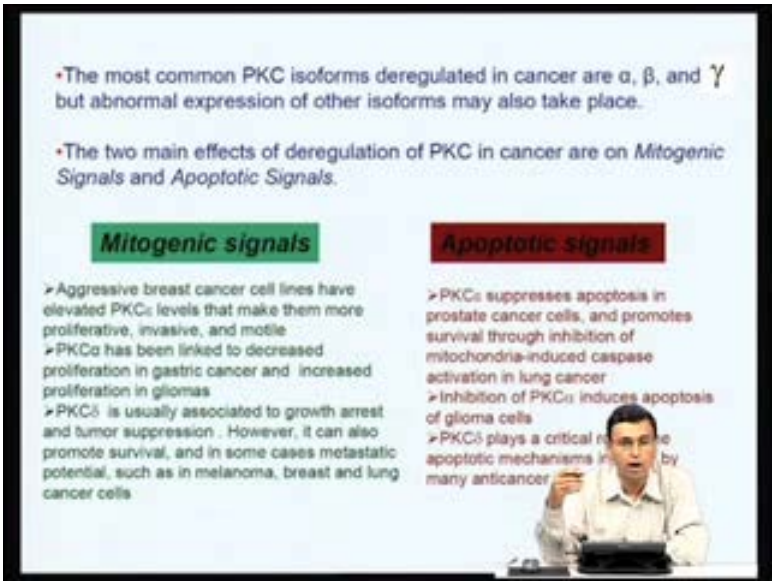

Similarly, the most common PKC isoforms deregulated in cancer are alpha, beta, and gamma– these are the classical PKCs, and all these will be when the… when these PKCs are misregulated or deregulated, it results in cancer. For example, the two main effects of deregulation of PKC in cancer are on mitogenic signals and apoptotic signals. Mitogenic signals, for example, aggressive breast cancer cell lines have elevated levels of PKC epsilon levels that make them more proliferative, invasive, and motile.

Similarly, PKC alpha has been linked to decreased proliferation in gastric cancer and increased proliferation in gliomas. PKC delta is usually associated with growth arrest and tumor suppression; however, it can also promote survival, and in some cases, metastatic potential, such as in melanoma, breast, and lung cancer cells. So, PKC deregulation plays a very important role in the activation of number of mitogenic signals.

Similarly, PKC epsilon is known to suppress apoptosis in prostate cancer cells and promote the survival through inhabitation of mitochondria induced caspase activation in lung cancer. Similarly, inhibition of PKC alpha induces apoptosis of glioma cells. Similarly, PKC delta plays a very important role in the apoptotic mechanisms induced by number of anticancer drugs. So, you can see, understanding the pathways by which protein kinase is activated can actually lead to development of inhibitors of this PKC, and see whether you can actually develop some novel anti-cancer compounds.

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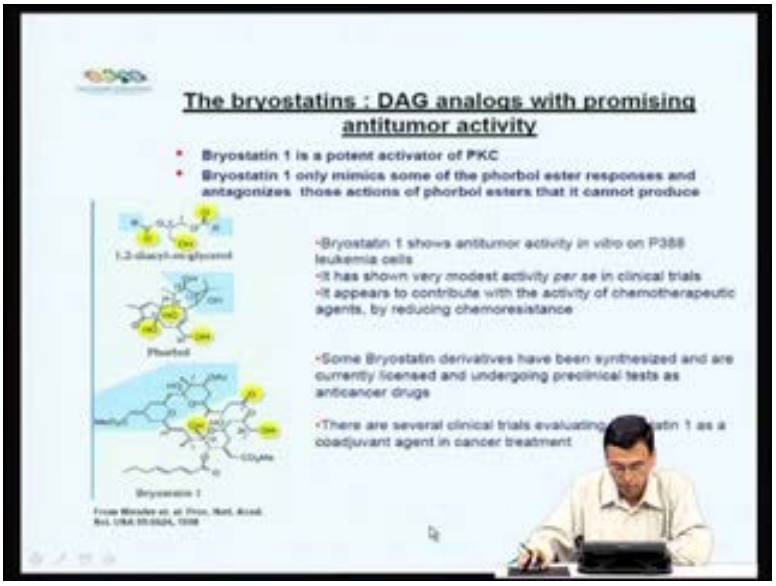

Just like a phorbol esters are very protein activators of protein kinase C, people have also come up with a number of analogs of diacylglycerol, and one such analog is called Bryostatin-1, which is also a structural analog of diacylglycerol, and Bryostatin-1 has also been shown to have anti-tumor activity on certain kind of leukemic cells and Bryostatin-1 is is a very potent activator of PKC.

The structure is shown here, taken from this particular paper, and Bryostatin-1 not only mimics some of the phorbol ester responses and antagonize those sections of phorbol esters that it cannot produce. So, variants of diacylglycerol– the structural analogs of diacylglycerol– like phorbol esters and Bryostatin are now been used, actually, to promote the anti tumor activity of protein kinase C, and in certain tumor cells, when you activate the protein kinase C using these molecules, it can lead to apoptosis or cessation of cell proliferation.

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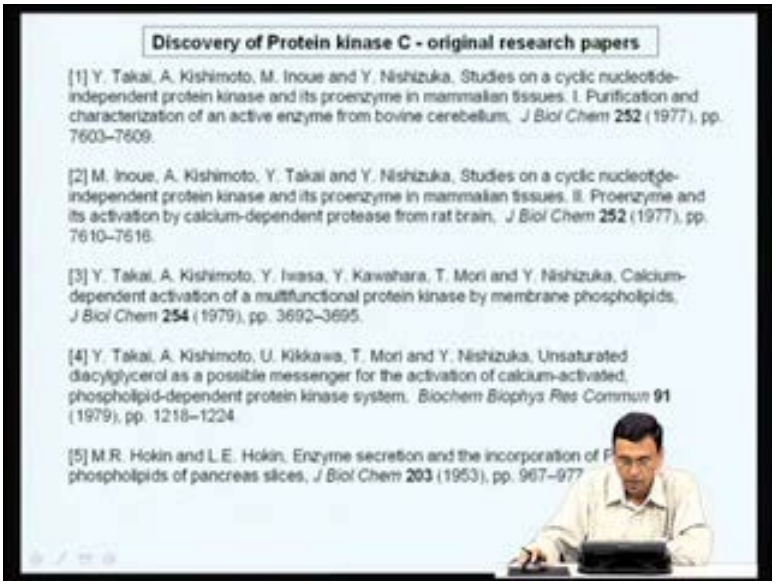

So, what I have listed in next few slides, this is some of the original research articles, especially in this slide, have listed the very important key research paper that actually lead to the discovery of the protein kinase C, by especially by the Nishizuka group, who are actually credited with discovery of protein kinase C. These, these are the first two JBC papers, which actually lead to the discovery of protein kinase C, way back in 1977.

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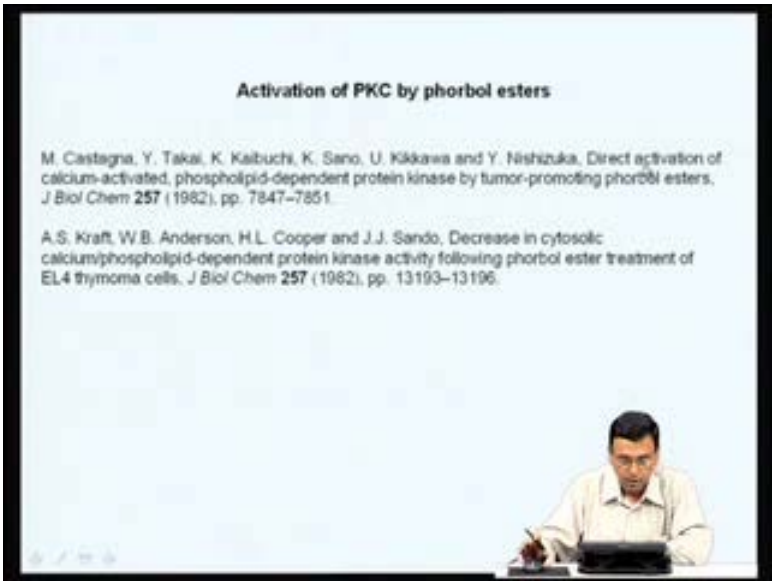

The activation of PKC by phorbol esters was first shown by in these two JBC papers, again by Nishizuka group in 1982.

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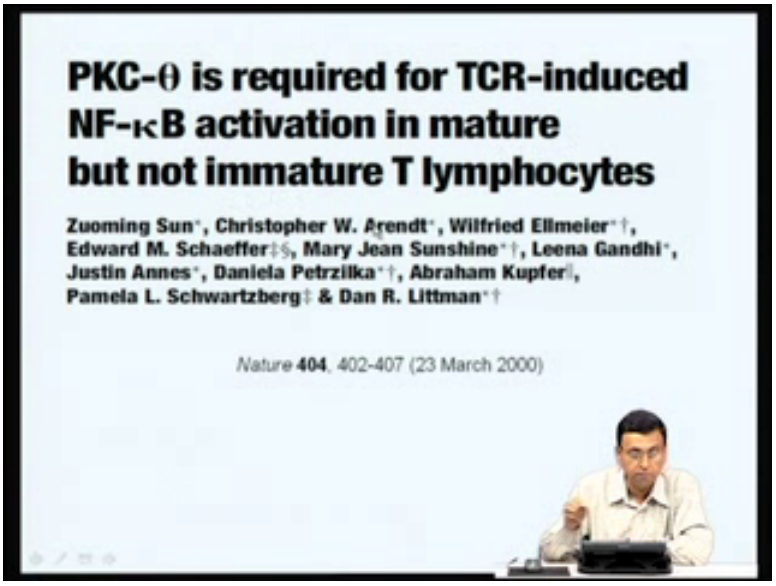

It is a very excited Nature, which actually shows how PKC theta is required for the T-cell receptor induced NF kappa B activation in mature, but not immature T lymphocytes.

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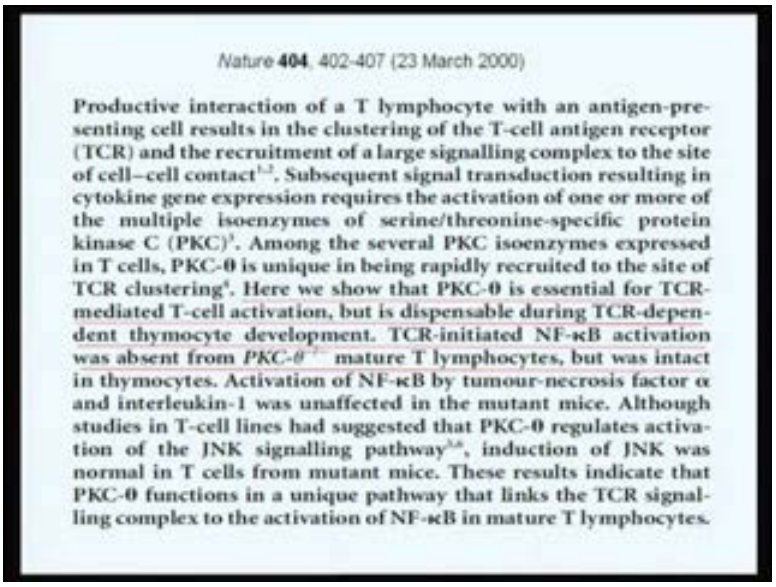

The paper clearly demonstrates that PKC delta is essential for T-cell receptor mediated T-cell activation, but is dispensable during T-cell receptor dependent thymocyte development, and T-cell receptor initiated NF kappa B activation was absent in PKC theta knockout mature lymphocytes, but was intact in thymocytes. So, using PKC theta

knockout mice, these also clearly demonstrated what is the role of PKC in T-cell signaling and T-cell function.

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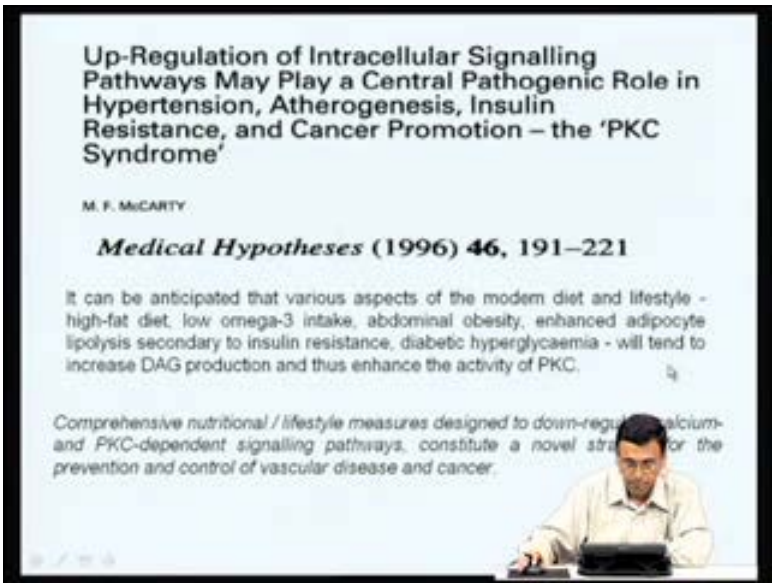

There is, again, a very interesting paper, which actually tells for what is called as PKC syndrome. How, by improper diet, you can actually develop what is called as PKC syndrome, and the the author very nicely says that it can be anticipated that various aspects of modern diet and lifestyle such as high fat diet, low omega-3 intake, abdominal obesity, enhanced adipocyte lipolysis secondary to insulin resistance, diabetic hyperglycemia– will tend to increase diacylglycerol production and thus enhance the activity of PKC.

So, if you now start eating high fat diet, this high fat diet, actually, produces a lot of diacylglycerol. This diacylglycerol can activate protein kinase C, leading into all kinds of problems. So, depending up on what diet you take can also influences whether protein kinase C pathway can may activated or not.

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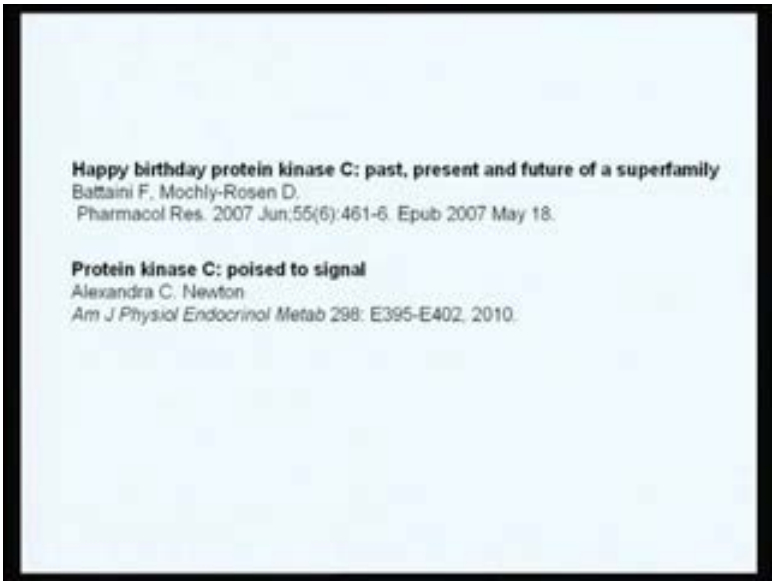

Two very important reviews to celebrates 25 years of discovery of protein kinase C– there is a very nice review article in Pharmacological Research– Happy birthday protein kinase C– a nice review of all the protein kinase C research that went on, and a more recent article in 2010– Protein kinase C: poised to signal– actually gives you overview of the importance of various PKC signaling pathways.

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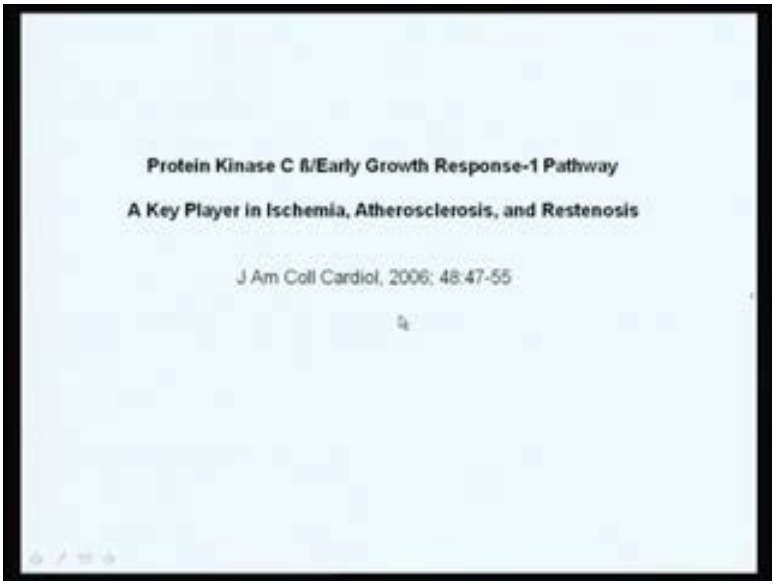

Again the… the role of protein kinase C in the early growth response pathway– how it can lead to ischemia, atherosclerosis, and restenosis, we discussed in this particular article.

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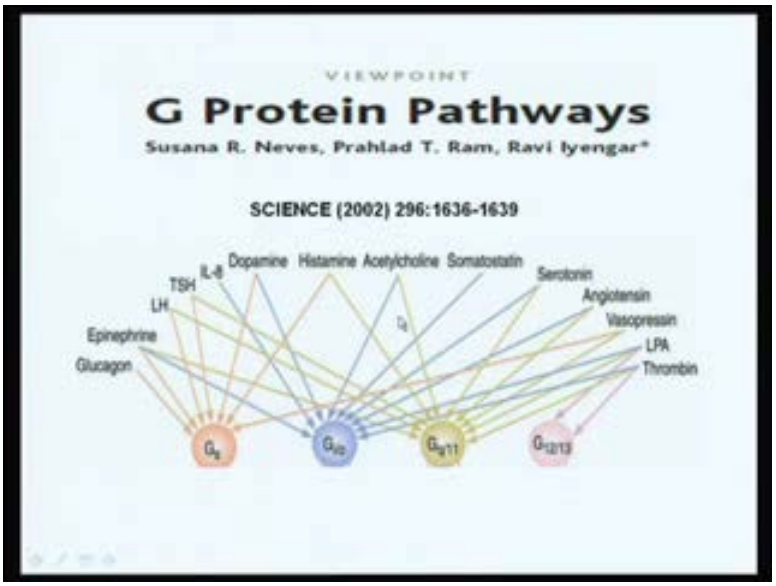

So, what I covered in the last three lectures is some of the aspects of the G-protein signaling pathways. I tried to cover the G s and G i pathways, but due to the paucity of time, I am not going to cover the remaining pathways, but I suggest to read you this excellent review in Science, which appeared in 2003, which very briefly discussed about the various G-protein– trimeric G-protein signaling pathways, and how this G-protein signaling pathway gives result in the regulation of number of physiological processes. I think I will stop here.