Eukaryotic Gene Expression: Basics and Benefits

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Lecture No. # 15

Signal Transduction Pathways – Introduction

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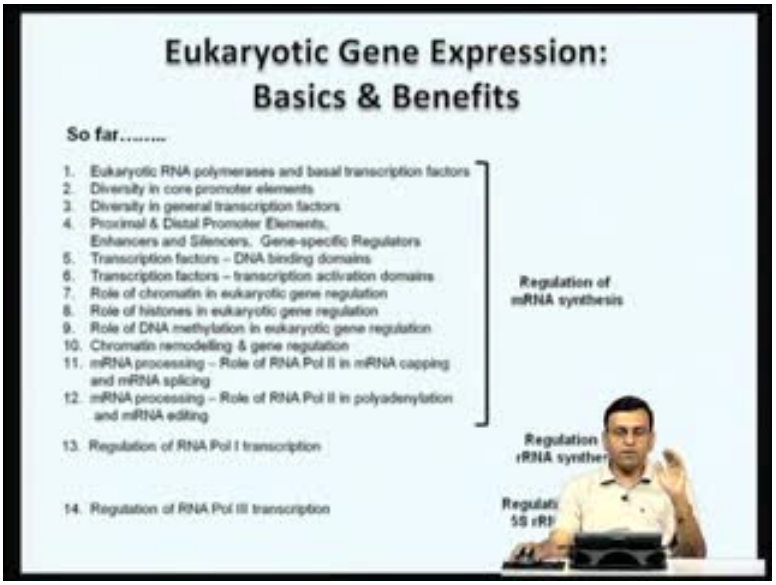

Today, we are going to discuss about signal transduction pathways. Now, why do we have to talk about signal transduction pathways? Now, so far, we have discussed how messenger RNA synthesis is regulated inside the nucleus. We took about 12 lectures to discuss various aspects of regulation of messenger RNA synthesis by RNA polymerase II. Then, we spent some time trying to understand how the ribosomal RNA synthesis is regulated by RNA polymerase I and synthesis regulated by RNA polymerase I.

Then, we discussed about the synthesis of transfer RNA and 5S RNA by RNA polymerase III inside the nucleus. So, all this time, we have confined ourselves to only the nucleus.

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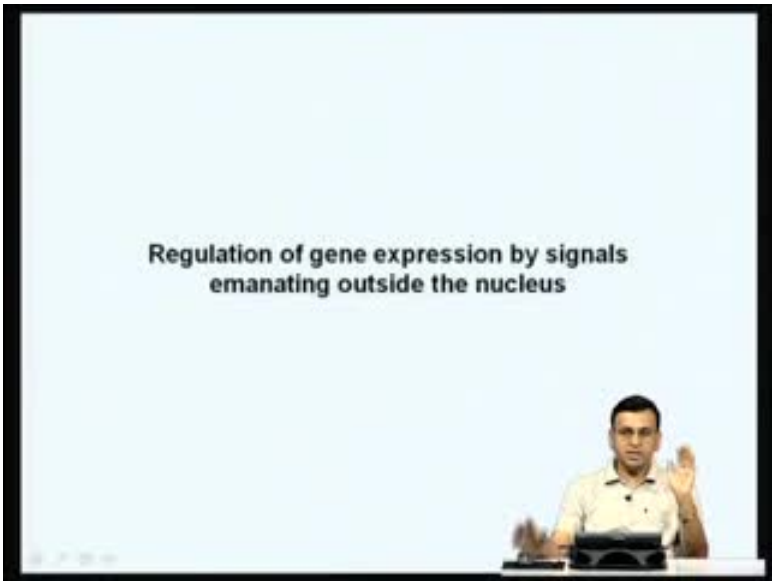

That is how gene expression is regulated inside the nucleus, how the chromatin environment influences the nucleus, epigenetic regulation of (( )) gene expression and so on and so forth. But, what we have to now do is to come out of the nucleus, and see how events which are taking place outside the nucleus, influence the gene expression inside the nucleus.

Because cells are not alone, cells are always either communicate with each other or they communicate with the environment. So, all the gene expression that was studied, so far, happening inside the nucleus, it has to be orchestrated by events taking place outside the nucleus.

So, unless we understand how it is these genes or how does the chromatin and the genes inside the nucleus communicate with the external environment, and how stimuli, either arousing extra cellular space or extra nuclear space, they influence gene expression, and that is what the crux of the entire regulation of eukaryotic gene expression. So, how signals emanating in the environment, as soon as signal emanating outside the nucleus, that is, in the cytoplasm, influence gene expression and regulate gene expression?

This is what the entire area of gene expression is all about. So, after understanding the basic mechanistic aspects of eukaryotic gene regulation, especially the three major forms of RNA, namely, transfer RNA, ribosomal RNA, messenger RNA, now, we will come

out of the nucleus and ask the question – how signals emanating outside the nucleus and in the cytoplasm influence gene expression changes?

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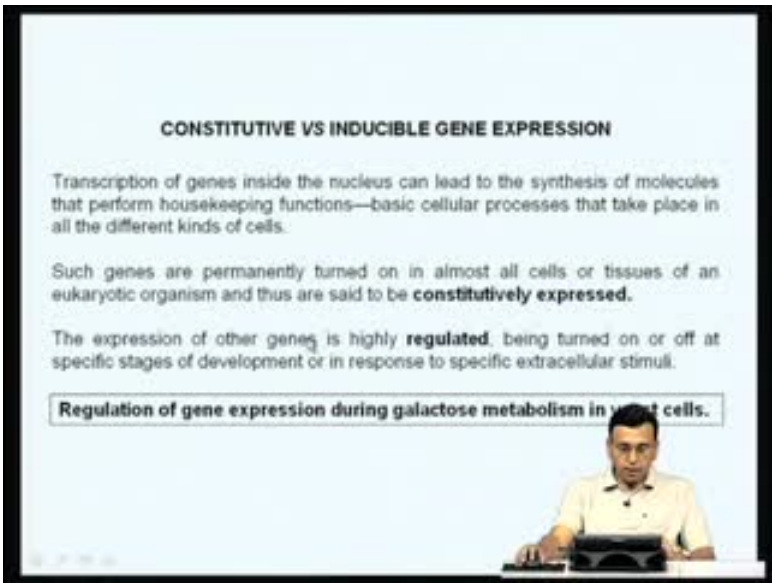

So, regulation of gene expression by signals emanating outside the nucleus is what is going to be focus of the talk, maybe in the next about 5 to 10 lectures. Now, when we talk about gene expression, there are basically two types of gene expression changes: one is constitutive gene expression and inducible gene expression.

So, the transcription of genes inside the nucleus can lead to the synthesis of molecules that perform housekeeping functions. When I say housekeeping functions, it means, the basic cellular processes that take place in all different kinds of cells.

So, for example, if we take an higher eukaryotic organism, whether it is a liver cell or a brain cell or a muscle cell, all the cells now have to make, derive, need energy for their metabolism and survival. So, basic energy metabolism pathways, that is, the enzymes involved in basic energy generating pathways and basic metabolic pathways like glycolysis, Kreb cycle, electron transport, oxidative phosphorylation, these things have to happen in all the cells, irrespective of whether it is a brain cell, or a liver cell, or a muscle cell. So, the genes, which are making these proteins or making the molecules involved in this protein, are turned on all the time, irrespective of all the cell types.

So, such kind of genes, which are required for basic aspects of, basic survival of the cell, or basic functioning of all the cell types, are known as housekeeping genes. So, such genes are supposed to be constitutively expressed, and they are turned on all the time in the cell, although their levels may be modulated in response to various cells stimuli.

Whereas, genes whose expression is cell type specific or tissue type specific or genes which are turned on only in response to certain a stimuli emanating outside the environment or outside the nucleus, they are called as inducible genes. So, we have two types of genes: one is constitutively expressed genes and inducible genes, and all the regulations takes place with these 2 kind of categories.

Let us give of a one simple example, before I go to high complex eukaryote, let us take a simple example from the simple eukaryotic like yeast, and ask the question – how does the yeast cells respond to a change in the carbon source in the medium? As you know, we normally grow yeast cells like Saccharomyces cerevisae if glucose is a carbon source, but suppose if you change the carbon source, some glucose to let us say galactose, what kind of gene expression changes take place?

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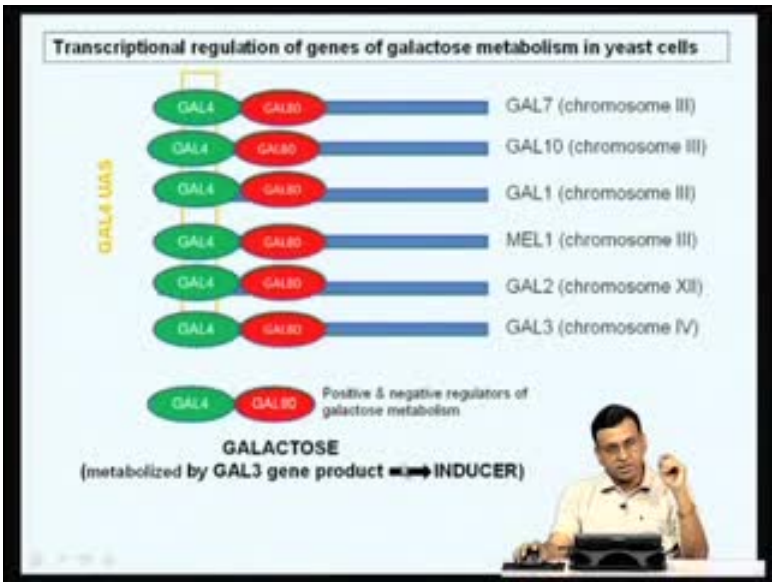

Now, so, what we will discuss, maybe in the couple of minutes, is how does transcription regulation takes place in response to galactose? If glucose is changed to galactose in the medium, how does the each cells respond to these kind of a change in the carbon source? Now, there are about half a dozen genes which are involved in galactose metabolism,

and each many of these genes are either present on the same chromosome, or they are present on the different chromosome. For example, the 3 genes have listed here, GAL7 is present on chromosome 3, GAL10 is also present on chromosome 3, whereas GAL1, which is also present chromosome 3

On the other hand, genes like MEL1, which is mellobiose metabolism, is present on chromosome 3, whereas the galact… GAL2 gene is present on chromosome 12, and the GAL3 gene is present on chromosome 4. So, the what I am trying to say here is that, there are about 6 genes involved in the galactose metabolism, and these genes are locate on of different chromosomes. So, the galactose metabolism has to be turned on in response to the presence of galactose in the medium. Then, these genes located on different chromosomes have to be coordinately switched on, and this is achieved by two transcription factors called GAL4 and GAL80.

Now, the GAL4 source is the positive regulator of gene expression. I think, in the previous lecture, when we started discussing about transcription factors, DNA binding domains, some transcription activation domains, we have discussed extensively the GAL4 protein of yeast, now, which has been extensively studied in one of the best eukaryotic transcription factors, which have been studied well. The GAL4 protein acts as a positive regulator of galactose metabolism, and the GAL80 protein, shown in red here, acts as a negative regulator of galactose metabolism.

Now, let us see how these genes are turned on. All these genes, despite being localized on different chromosomes, in their promoters, contain an enhancer element known as GAL4 upstream activation sequence, shown as yellow here. So, this upstream activation sequence is specific for all these genes involved in galactose metabolism, although it will be some minor variations.

Now, what happens, as long as we grow yeast cells in the absence of galactose, the GAL4 and GAL80 interact with each other, and they bind to the promoter element, especially the GAL4 UAS, and inhibit the transcription of the galactose metabolism genes; which makes perfect sense, because there is no need to turn on the genes involved in galactose metabolism, when there is no galactose in the medium.

The moment you add galactose to the medium, or the moment of glucose is replaced by galactose as carbon source, the GAL3 gene metabolizes the glucose, and this metabolite

of galactose now acts as an inducer, and this inducer now prevents the binding of GAL80 to the… now interaction of GAL80 with GAL4, and as a result, now GAL4 acts as a positive regulator and turns on all the genes involved in galactose metabolism. So, you can see, by simple change in a carbon source from glucose to metabolism, the gene expression inside a nucleus is turned on or turned off, and glucose is there, the GAL4 and GAL80, too, interact with each other, and all the genes involved in galactose metabolism are not expressed when we change the galacto… the carbon source to galactose, because cells now have to metabolize galactose.

This is signal is passed on to the nucleus, and all the genes involved in galactose metabolism is turned on by inhibiting the interaction between the GAL80 and GAL4, and GAL4 now acts as positive regulator and turns on all the expression of the genes. So, this is one simple examine example just to illustrate, although we have studied, so far, all the gene expression, organization of genes, and expression of the genes are nucleus, all these things would happen in coordination with the change that is taking place outside the nucleus. And one simple example is the how, for example, yeast cells respond to changes in the carbon source.

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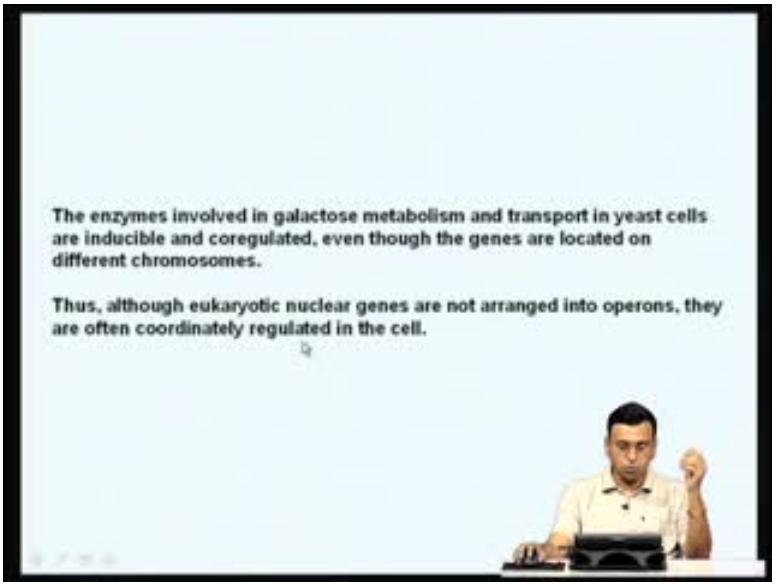

Now the enzymes of involving galactose metabolism and transport in yeast cells are inducible and co-regulated, even though the genes are localized on different chromosomes. So, this is unlike what we are seeing in e coli, where all the genes

involved in a particular metabolic path were often present together, and organized in the form of operons. Now, in the higher eukaryotes, and even in simple eukaryotes, this kind of operons seldom exist.

But, despite being localized on different chromosomes, the genes of a particular metabolic pathway are coordinately regulated, because they all have common cis-acting elements, to which a common trans-acting element may have to bind and activate the expression of a number of genes. All these genes are involved in particular metabolic pathway.

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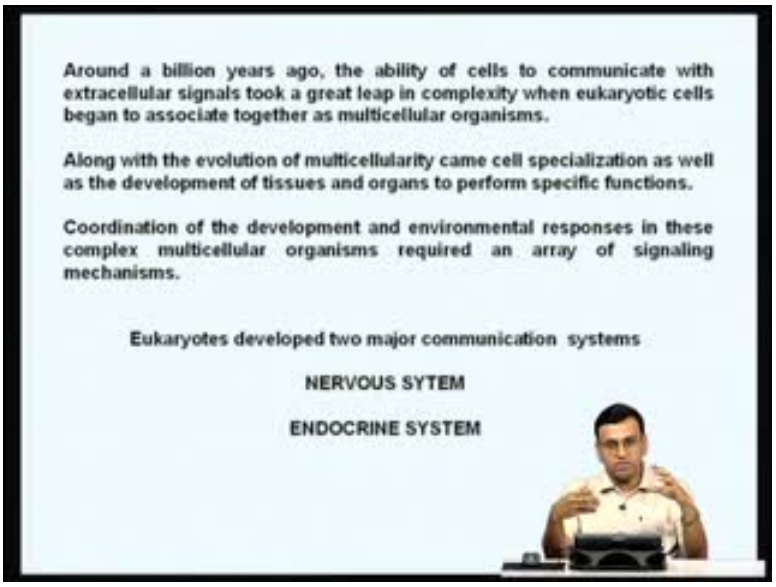

So, although eukaryotic nuclear genes are not arranged into operons, they are coordinately regulated in the cell, and I given you, the galactose metabolism is one example. There are umpteen number of examples, as we, as we will discuss more and more about it.

Now, let us come to complex eukaryotes. Now, the simple eukaryotes like yeast are single cells. So, most of the time, the major communication they have to do is with the extracellular environment. But around a billion years ago, the ability of cells to communicate with extracellular signals took a great leap in a complexity, when eukaryotic cells became…, began to associate together and started evolving into multicellular organisms. So, it became clear, because when these cells started coming together and started for organize in the form of tissues, various cell types, it became

necessary for them not only to interact with environment, but they also have to interact with each other. So, cell communication, as well as communication of cells with the external environment, became very important.

So, along with this evolution of multicellularity came cell specialization. Whereas in the case of simple eukaryotes like yeasts, you only have a single cell type, but as the eukaryotes became multicellular, each cell became a specialized (( )) you have… there are cells which are specialized for carrying out a cardiac function, cells which would be specialized for carrying endocrine function, cells became specialty becomes nervous system, and so on and so forth.

So, as this cell specialization, as well as development of various tissues organisms became evident, it became much more necessary to develop complex communication systems and complex regulatory systems to coordinate all these activities together, so that the organism can function in a coordinated manner. So, coordination of the done develop of the development and environmental responses in these complex multicellular organisms required an array of signalling mechanisms.

So, the signal transduction pathways or the signalling mechanisms, that is, the signals, which regulate gene expression inside nucleus, became more and more complicated. At least 2 important communication systems were developed in the multicellular eukaryotes: one is the nervous system, another is the endocrine system. So, the gene expression that is taking place inside nucleus have now to respond to the changes taking place outside the cell, through, at least, through this major communication network, such as the nervous and nervous system and the endocrine system.

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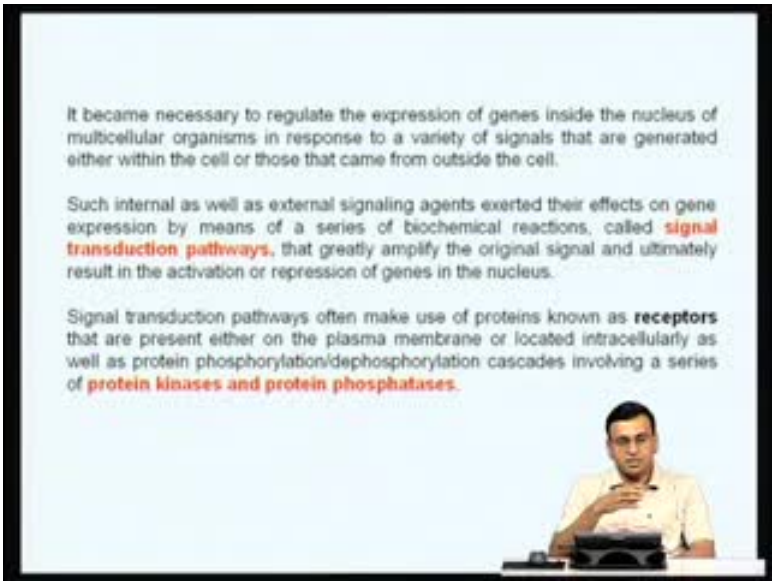

So, it became necessary to regulate gene expression inside the nucleus of a multicellular organisms, in response to a variety of signals that are generated, either within the cell, or those that came outside the cell. This what we have been telling, now, exerted their effects of gene expression by means of a series of biochemical reactions called signal transduction pathways. These signal transduction pathways greatly amplified the original signal, and ultimately resulted in the activation or repression of genes in certain nucleus.

So, this is what the need for a complex signal transduction pathway is a necessity, because as the simple eukaryotes like yeast became evolved into a multicellular or complex organisms, the… in order to respond to these external cues, external signal stimuli, cells developed complex signal transduction pathways, so that the signal can be properly amplified and channeled through a specific pathway, ultimately leading to activation or repression of specific genes. And these signal transduction pathways often make use of proteins known as receptors, which act as a entry point, and if the receptors, which actually interact with the specific signalizing molecules, and these receptors are either present on the plasma membrane or they are present inside the cells.

The entire signal transduction pathway is governed by a series of phosphorylation and dephosphorylation events, which are basically governed by protein kinase And protein phosphatases. So, what we are going to now begin to understand is that the as organization became multicellular, it became necessary to evolve complex signal

transduction pathways so that gene expression can be either activated or repressed in response to specific environmental signals, and these signals can either communicate… the, these signals can either act at the level of cell membrane or they can diffuse into the cytoplasm, and interact with these signalling molecules, interact with specific receptor molecules, and then, ultimately, through a cascade of phosphorylation-dephosphorylation events, ultimately, this entire signal transduction pathway culminates in the activation or repression of transcription of specific genes.

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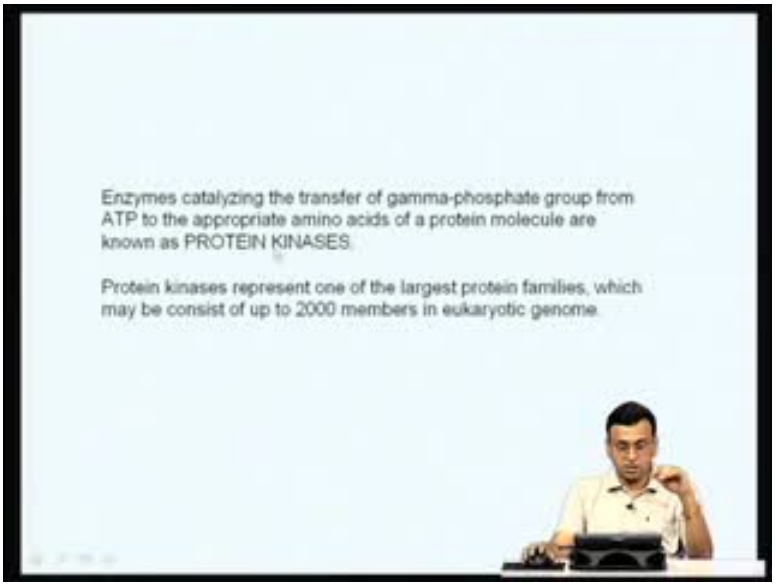

Now, the enzymes, which catalyze the transfer of a gamma phosphate from ATP to specific protein molecules, are known as protein kinases. By this time, I am sure, all of you are aware of what protein kinases are, because we studied extensively the how phosphorylation of histones regulate gene expression, and so on and so forth, in the previous lectures.

These protein kinases play a very important role in the signal transduction pathway as well. So far, in the earlier lectures, we studied how phosphorylation of histones can regulate gene expression inside the nucleus. Similarly, the protein kinases, even outside the nucleus, by specifically phosphorylating and specific receptor molecules, as well as other proteins, can ultimately influence gene expression.

The protein kinases represent one of the largest families, which may consist of up to 2000 members in a eukaryotic genome, and even as I speak today, we still do not

understand the exact physiological significance as well as mechanism of action of many protein kinases inside the eukaryotic cell.

So, one of the major challenges of the twenty first century is to understand how these various signal transduction pathways coordinate with each other, they work with each other, interact with each other, ultimately leading to activation of a specific gene expression program. This is been one of the major challenges, and we still have not achieved success in many of these signal transduction pathways.

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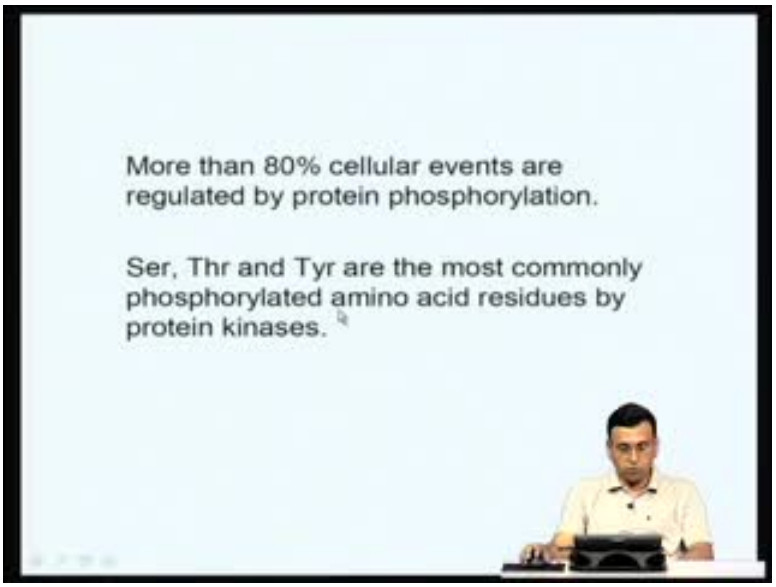

So, more than 80 percent of the cellular events are regulated by protein phosphorylation, and the 3 major residues in a protein, which can be modified are, serine, threonine, and tyrosine, and these are the three residues which are targeted by the protein kinases, which we have already studied in the case of the histone gene regulation.

But the regulation of gene expression by phosphorylated histones primarily involves serine and threonine residues, whereas, when we come to signal transduction pathways, the tyrosine plays a very important role, and, in fact, there are a group of kinases known as tyrosine receptor kinases, which play a very important role in a number of signalling pathways.

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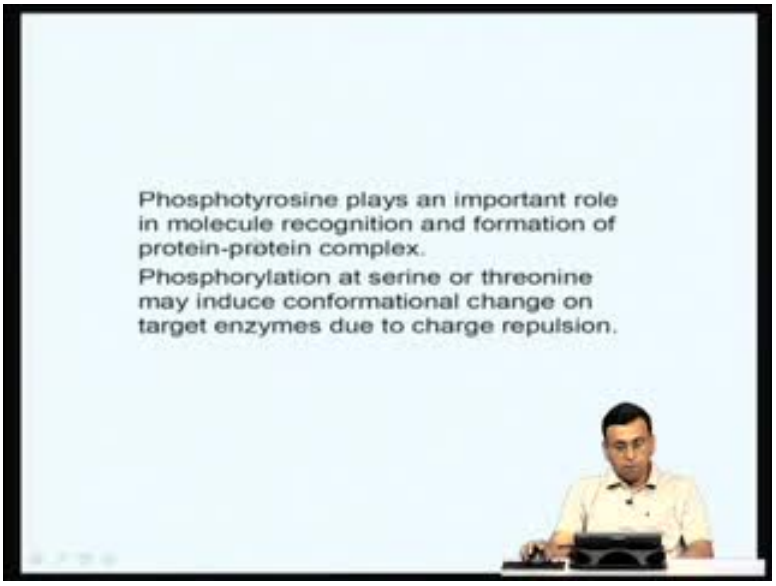

Phosphotyrosine plays an important role in molecule recognition and formation of a protein-protein complex, and phosphorylation at serine or threonine induce, often, conformational changes on target enzymes, due to change in the repulsion. The charge-charge interaction lead to repulsion, and result in the conformational change, and this triggers a cascade of events, ultimately culminating in activation or repression of specific target genes.

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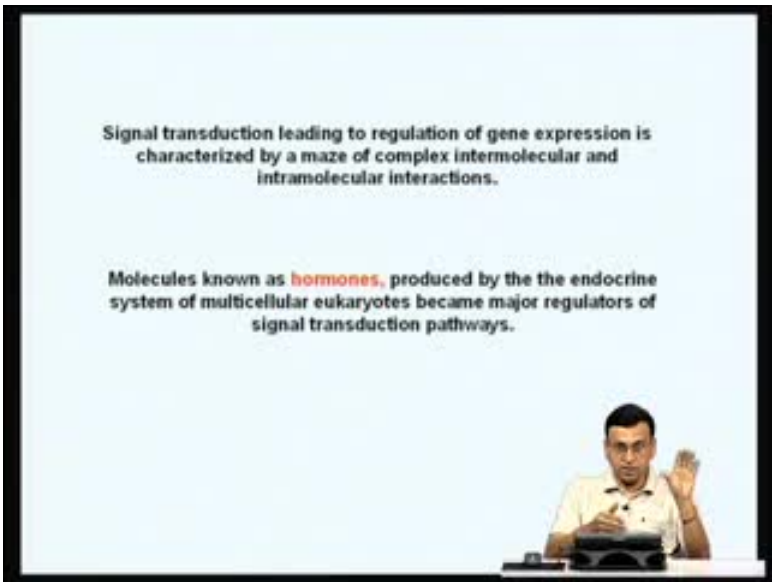

So, signal transduction, leading to the regulation of gene expression, is characterized by a maze of complex intermolecular and intermolecular interactions. I am trying to prime you to the complexities you are going to face at the later stages of this lecture series, because as I said, these signal transduction mechanisms have now become very complicated, and as I speak, we still do not understand the exact mechanisms that are involved on the interplay of the various factors, ultimately leading to the activation of a specific gene expression program. So, not only each signal transduction pathway operates and activates a gene; often, a single gene– the activation or a repression of a single gene depends on multiple signals and multiple signal transduction pathways.

So, a lot of research is now going on, to understand how these signalling molecules or how these signalling pathways communicate with each other, and as a result of this complex inter molecular communication as well as intra molecular communications, how specific genes are either activated or repressed.

What we will now study and focus, primarily, is, at least the initial part of this lecture series, is to understand the role of the endocrine system on the role of hormones; how hormones, which are very important chemical messengers that have evolved in complex multicellular eukaryote molecules, how these hormones ultimately activate or repress the expression of target genes. As we all know, hormones are primarily produced by the endocrine system of multicellular eukaryotes, and these hormones became very important players in the regulation of gene expression through specific signal transduction cascades.

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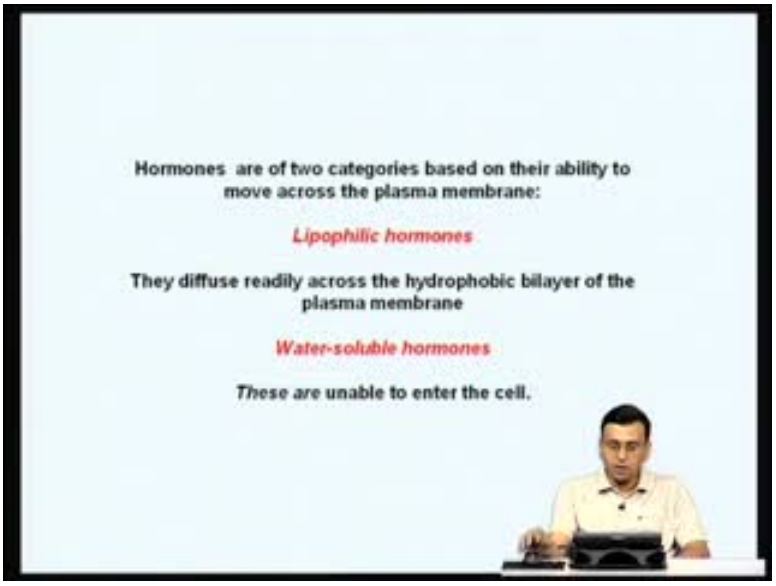

Now, so when we talk about hormones, which are chemical cell messengers which are secreted by specific endocrine organs in the body, and they then move to another place and then exert their effect– this is what the simple definition of a hormone, which most of you are studied at a very early stage of your education.

So, these hormones, which are secrete by our endocrine system of the body, there are primarily of 2 categories: one are called the lipophilic hormones, and these lipophilic hormones are capable of moving because of their hydrophobic nature. They can simply diffuse across the cell membrane or the plasma membrane and enter the cell, and inside the cell, or inside the cytoplasm, or in the nucleus, they can interact with specific receptor molecules, ultimately to the activation or repression of specific target genes.

In contrast, there are also what are called as water soluble hormones, which could be either small molecules, or it could be many polypeptide hormones, which are hydrophilic, and because of their inability to cross the lipid membrane, these water soluble hormones primarily interact with receptors which are present on the cell surface, and then, this interaction of the ligands with the cell surface receptors ultimately triggers a series of events, ultimately culminating in the activation or repression of the target genes.

So, there are 2 kinds of signal path, 2 major kinds of signal transduction pathway, as well as hormones are concerned, one group of molecules, they simply diffuse into the

cytoplasm through the plasma membrane, interact with specific intracellular receptors, and activate the expression of, or repress the transcription of various genes. There are molecules which are unable to cross the plasma membrane barrier, and as a result, they interact specifically with membrane receptors, and this interaction of the membrane receptors with these specific ligands triggers a series of events, ultimately leading to activation or repression of specific target genes.

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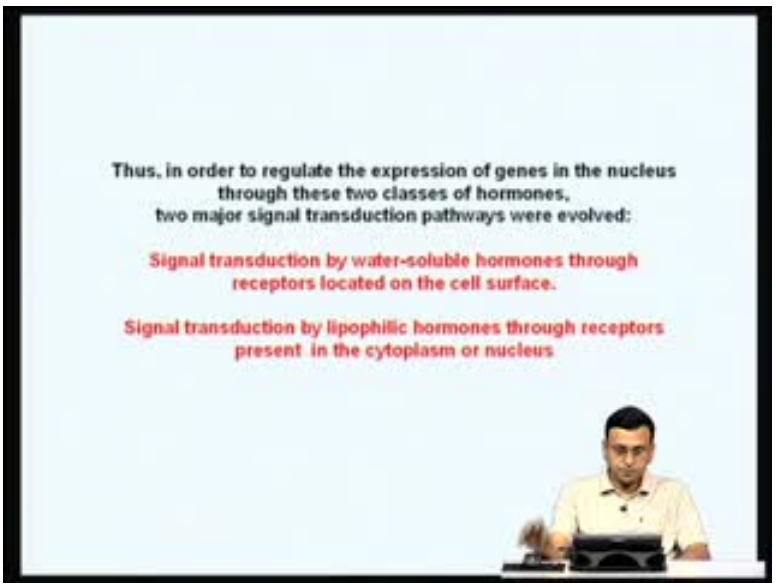

So, in order to regulate the expression of genes in the nucleus through the decline through these 2 class of hormones, 2 major signals transduction pathways were evolved. This is what we just discussed, just now. Signal transduction by water soluble hormones is through receptors located on the cell surface, and signal transduction by lipophilic hormones is through receptors present in the cytoplasm or nucleus.

So, one is called as extracellular receptor, membrane receptors, another is the intracellular receptors, which can be present either in the cytoplasm or the nucleus. Now, so, this a cartoon, which basically tells you what I have told you so far.

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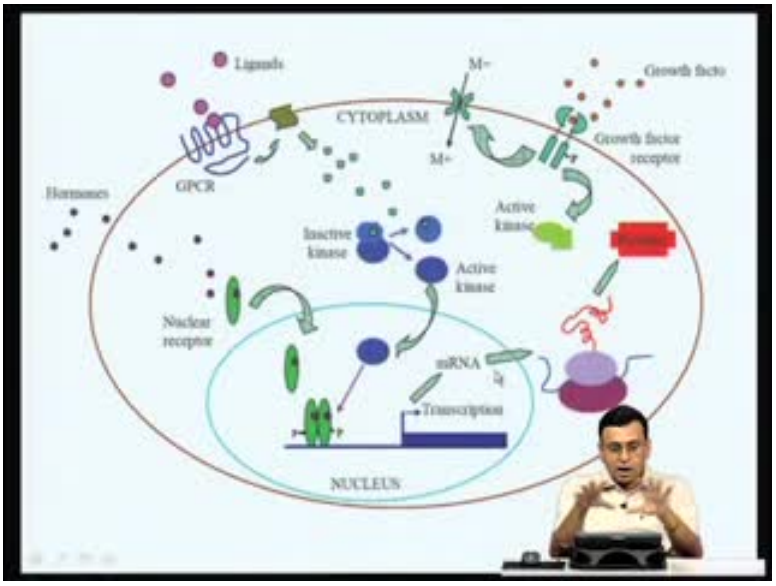

For example, there are, for example, certain hydrophobic molecules. These can be either small ligands or they could be peptide growth factors. Both these categories, they interact with specific receptors, which are present on the membrane, and these receptors are of all often known as transmembrane proteins, because they span both, they communicate with both extracellular side as well as the intracellular side of the cytoplasmic side, and they traverse across the plasma membrane, often through characteristic domain.

We will talk about it little bit later. So, these, using this kind of a transmembrane proteins, the molecules– either small ligands or peptide growth factors or a poly peptide growth factors, communicate with specific receptors present on the cells; like in one case, these are called as G-protein coupled receptors or GPCRs, or it could be something like growth factor receptors, which actually serve as receptor tyrosine kinases. We will discuss this in later.

These kinds of receptors communicate with these small molecules, and then transmit the signal through a series of phosphorylation relay mechanism, ultimately leading to the phosphorylation or modification of specific transcription factor, leading to transcription of specific messenger RNAs and synthesis of specific proteins, as shown here.

In direct contrast, there are certain molecules, especially molecules like steroid hormones, because of the lipophilic nature they can readily cross through the membrane barrier, and then they interact with specific receptors, which can be either present in the

cytoplasm or they can be present in the nucleus, and then the receptor comes inside, often they dimerize, and then bind to specific sequences of target genes, leading to transcription and synthesis of specific proteins.

So, the whatever I discussed so far, I shown in the form of cartoon– how hydrophilic ligands interact with specific membrane receptors, and how to hydrophobic molecules simply diffuse through the plasma membrane, interact with specific intercellular receptors. Ultimately, the both the pathways converge into activation or repression of specific target genes.

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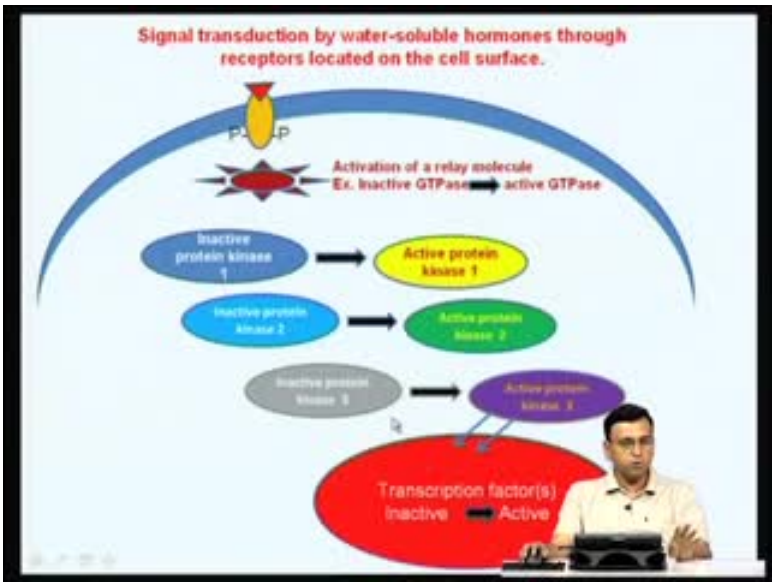

Now, made a small cartoon to explain to you how signal transduction by water soluble hormones take place through receptors present on the cell membrane. Let us assume, this is the plasma membrane of a cell, and you have a specific transmembrane receptor, which is capable of communicating both outside the cell as well as inside the cell.

So, once a hydrophobic, once a water soluble molecule– it could be molecules like a epinephrine, or it could be something like an epidermal growth factor; it could be insulin; it could be glucagon; we will discuss some of these examples in great detail as we go along.

So, either macromolecules like, huge molecules like the polypeptide growth factor, or it could be a small molecules like epinephrine, nor epinephrine, so on and so forth, they

can interact with some of the specific cell surface receptors, and as a result of this receptor-ligand interaction, invariably, it results in a conformational change in the receptor molecule, leading to auto phosphorylation of the receptor.

That is why, many of these receptors are known as protein kinases, but in there are exceptions were phosphorylation is not a major mechanism. So, ligand-receptor interaction, ultimately, leads to the activation of certain relay molecules, and one of the most common relay molecules, which are used in these signal transduction pathways, are what are called as GTPases or G proteins.

Now, these G proteins are basically enzymes, which are capable of hydrolyzing GTP to GDP. So, one of the major signal transduction methods involving the signal transduction or water soluble hormones is, when the hormone interacts with receptor, it results in the activation the of a GTPase, rising to hydrolysis of GTP to GDP.

This GTPases are capable of choosing their partners depending upon their past association with either GTP or in a GTP state, and these GTPase a usually inactive state when they are bound to GDP, and they become activated when they bound to GTP, and the GTP (( )), they go and associate or activate specific protein kinases, and I have shown here, the one of the important downstream cascade that happens at, through this relay mechanism involving GTPases, is the activation of an inactive protein kinase to a active protein kinase, and often, the signal transduction pathways have this kind of a activation and inactivation of the series of protein kinases A.

As I have shown here, protein kinase 1, which is inactive form, gets activated protein kinase to active protein kinases 1, and this active protein kinase 1, in turn, phosphorylates another protein kinase 2, which then becomes active, and this again can go and phosporylate another inactivated protein kinase, and then convert it to an active form, and as a result of this series of phosphorylation cascades, the ultimately, the final protein kinase now goes inside the nucleus, which I have shown in the red here, and activates a specific transcription factor by phosphorylation, leading… and these transform transcription factors can then go, either associate with histones acetyltransferases, or they can associate with histone deacetlyases, and so on and so forth, leading to either activation or repression of transcription.

So, this is one of the major mechanisms by which molecules, which cannot enter the cell, inter… but interact with specific membrane receptors, activate transcription in the nucleus through a series of enzymes and protein kinases, leading to phosphorylation-dephosphorylation mechanisms, ultimately resulting in the activation or repression of specific genes inside the nucleus.

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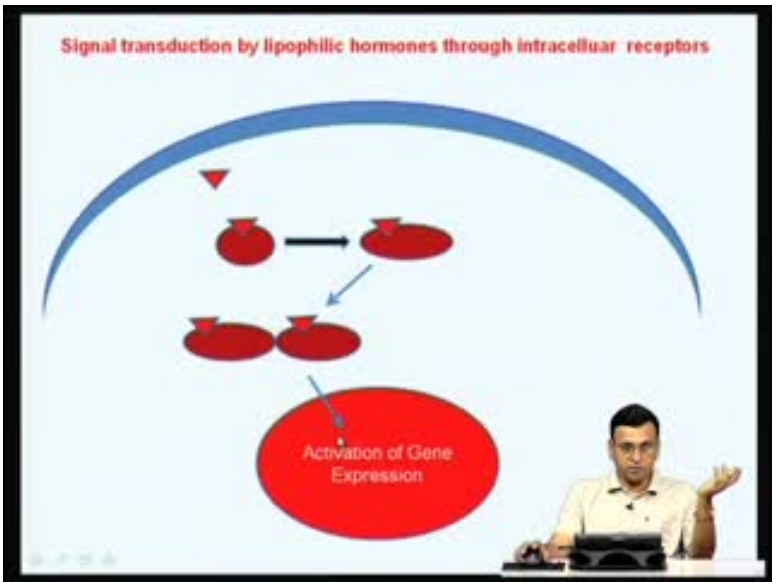

Now, in the case of those molecules, especially the hydrophobic molecules like cell hormones, and so on and so forth, these molecules, unlike the previous ones, can actually enter the cell, because they can just diffuse across the plasma membrane; and once they enter the cell, they interact with specific intercellular receptors, as I mentioned earlier.

Some of these receptors may be in cytoplasm, or some of the receptors may be present inside the nucleus. Now, once this ligand interact with this receptor, usually, this ligand-receptor interaction results in the conformational change inside the receptor, and as a result of this conformational change, the molecule now can often dimerize. They can either form homodimers or heterodimers; that was example for both kinds, and these dimerize, now go inside the nucleus, bind to specific response element of genes, and activate the expression of various genes.

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So, the 2 cartoons have show for shown, so far, is the 2 major significant transduction pathways involving receptors acting either the cell membrane level or receptor acting either at the through intercellular receptors.

Now, things are not as simple as what I have mentioned here. The signal transduction pathways are very complicated. Often, numerous signals are required to turn on or turn off the expression of a specific gene, and each of these signals is transmitted back to the gene by a separate regulator pathways.

Because cells are not responding to just 1 signalling molecule cells are cell substitution our body often bombarded with the multiple signals. So, often, in a given cell, it often is bombarded with multiple signals, and multiple signal transduction pathways are getting activated at any given time, in a any cell type or in tissue type, and so, the ultimate activation or repression of genes, often, did is the sum total of all these activation or repression of multiple signal transduction pathways, and often, a single gene may be activated by molecules or signalling molecules coming from multiple signalling pathways.

So, multiple signalling molecules and multiple transcription factors often act together, leading to synergistic activation or repression of transcription of specific genes. So, we will discuss now specific example, as we go along, to understand how complicated the entire gene expression regulation is, especially, when you now come out of the nucleus

and start asking the question– how events taking place outside the nucleus, outside the cell, influence gene expression programs inside the cell?

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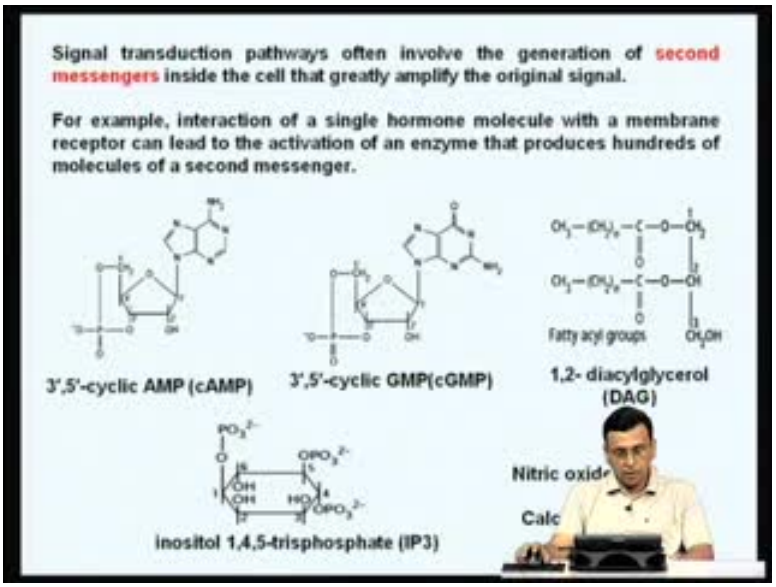

One of the important thing that happens in the signal transduction pathways is the generation of second messengers. The ligand of the hormone, which interacted with the, with the receptor and stayed outside, is the primary messenger, and often, the interaction of this hormone with the ligand, results in the generation or what we called second messengers, and if these second messengers ultimately communicate, they take the signal further down.

The one of the important significance or advantage of the second messenger is that just 1 hormone molecule enter the receptor. This single molecule interaction ultimately results in this generation of hundreds or even thousands of second messenger molecules. So, the signal gets tremendously amplified, and that is how even very low doses of a hormone is able to influence a gene expression a big way.

Many of these hormones need not be synthesized in the huge amounts. Even very small amounts of hormones is able to exert their physiological responses, because they can ultimately act through, or they ultimately act through these second messengers. So, a interactions happening at the cell membrane, even at a single molecule level, even a single molecule interaction with single cell surface receptor, can generate hundreds, or even thousands, of second messenger molecule until the signal gets amplified, leading,

ultimately, the activation of number of a huge amount of a target genes. The magnitude of activation of target gene expression is enhanced. For example, interaction of a single hormone molecule with a membrane receptor can lead to the activation of an enzyme that produces hundreds of molecules of a second messenger.

We will discuss in detail how exactly this takes place, but remember, the production of a second messengers is a very important adaptation that eukaryotes have evolved, whereby the original signal gets dramatically amplified, because all that hormone has to do is to activate an enzyme, so that this enzyme can now produce a huge number of these second messenger molecules, and they, this amplified signal ultimately results in the a dramatic increase or decrease in the gene expression.

Some of the common second messengers are listed here, like they have what is called the cyclic AMP; we have cyclic GMP; we have Diacylglycerol; we have inositol triphosphate, nitric oxide, calcium, these are all the various second messengers that are generated in the cell by various hormone-receptor interactions. Each one of them, ultimately, go and activates specific set of genes, which ultimately results in the synthesis of specific proteins or other macromolecules, and thus gets translated into a specific physiological response.

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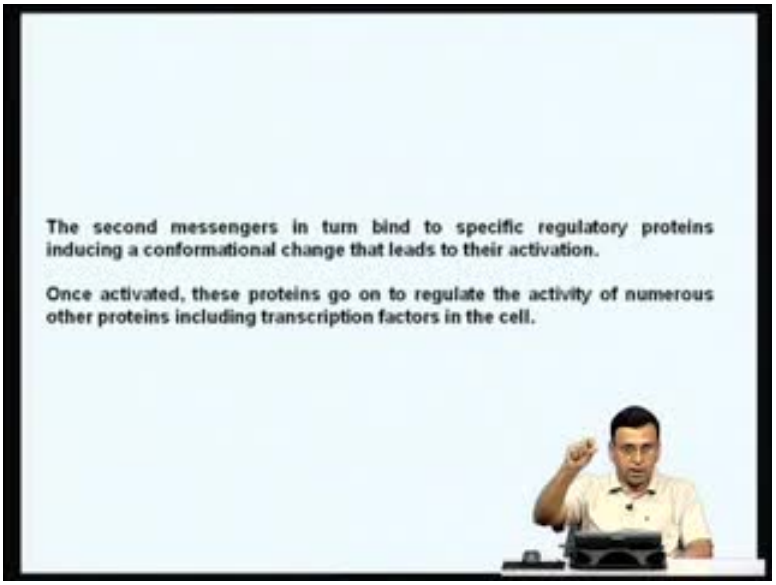

The second messengers that that are generated in response to a hormone-receptor interaction, in turn, bind to specific regulatory proteins, inducing the conformational

change that ultimately leads to their activation. And once activated, these proteins go on to regulate the activity of a numerous other proteins, including transcription factors inside the cell.

So, this is one of the basic mechanism that I would like to highlight– how molecules, either intact in specific cell surface receptors, can lead to the generation of specific second messenger molecules, and even 1 molecule interaction with 1 receptor molecule can activate, as a result of this phosphorylation cascade, a, an enzyme, which can generate a very high number of second messengers, and thus, the original signal gets dramatically amplified.

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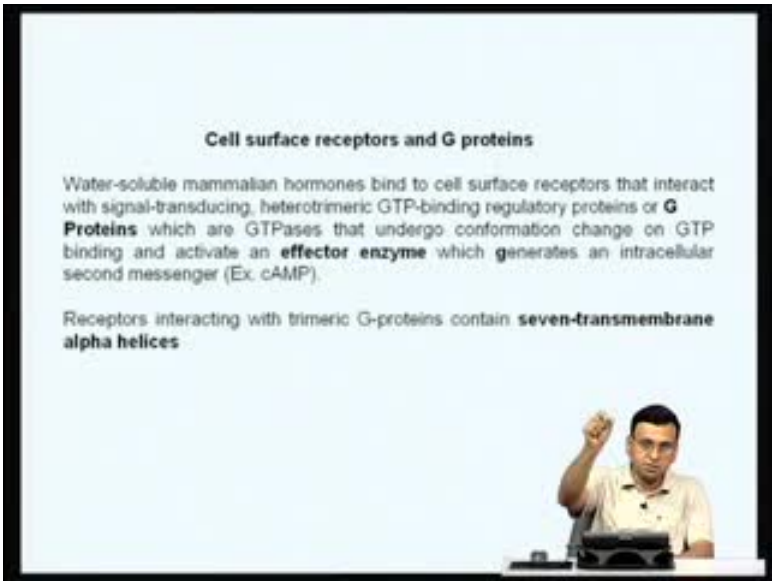

So, let us now spend some time to understand how cell surface receptors activate gene expression. We going to begin with what are called as G proteins, and then we will get into other kinds of cell surface receptors, and ask the question– how various, what are the various kinds of cell surface receptors present in the signal transduction pathways, and what kind of molecules are involved in the activation of these signalling molecules or signalling pathways.

Now, water soluble mammalian hormones bind to cell surface receptors that interact with signal-transducing heterotrimeric GTP-binding proteins, or G proteins, which are nothing but GTPases that undergo conformational change on GTP binding, and activate an effector enzyme, which generates an intracellular messenger called cyclic AMP. So, I am

going to begin with one of the major signal transduction pathways that operate in eukaryotes.

This pathway primarily involves the binding of a hormone, which is basically a water soluble hormone, so that it cannot enter the cell. So, interaction of a water soluble hormone with its receptor results in the activation of a GTPase, and this GTPase usually exists in the form of a trimeric protein, and as long as a protein is present as a trimer, it is inactive, and once it is activated by the hormone bound receptor, then the GTPase are dissociates, and one of the subunits now become active. That, in turn, now goes and actives an enzyme called adenylate cyclase, and this adenylate cyclase now starts producing cyclic AMP, and cyclic AMP now acts as a second messenger and initiates a phosphorylation cascade, ultimately results in the activation or repression of specific target genes, or it can also result in the activation or inactivation of specific enzymes in a particular metabolic pathway. So, what we are now going to focus in this lecture, is about a class of receptors called as trimeric G proteins, which basically contains seven-transmembrane alpha helices.

So, today's class, were going to now discuss about 1 particular signal transduction pathway, which ultimately involves the production of cyclic AMP, and we are going to now study how cyclic AMP is produced when a specific hormone molecule interacts with the specific cells of a receptor, and what does the cyclic AMP do later.

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This kind of a G protein-coupled receptors or the GPCR signalling, is a very robust mechanism of signal transduction pathways that operate in the living cells, especially eukaryotes, and a number of molecules, as well as big molecules like polypeptide hormones, make use of this GPCR signalling pathways to activate the repression of enzymes or even transcription factors.

Some of the molecules which activate gene expression programs you through this GPCR signalling are listed here– this includes certain amines, nucleotides, eicosanoids, certain lipid molecules, or lipid derived in derivatives, peptide hormones, proteases like thrombin, many glycoprotein hormones like the LH, follicle stimulating hormone, human chorionic gonadotropin, thyroid stimulating hormone, all these big molecules, as well as calcium, glutamine, gamma amino gamma aminobutyric acid, all these molecules activate gene expression programs, or activate enzymes or inactivate enzymes using this GPCR signalling pathway, and again, a reference, a nice review which gives a very good overview about this–the mechanism by which all these molecules activate through the GPCR signalling.

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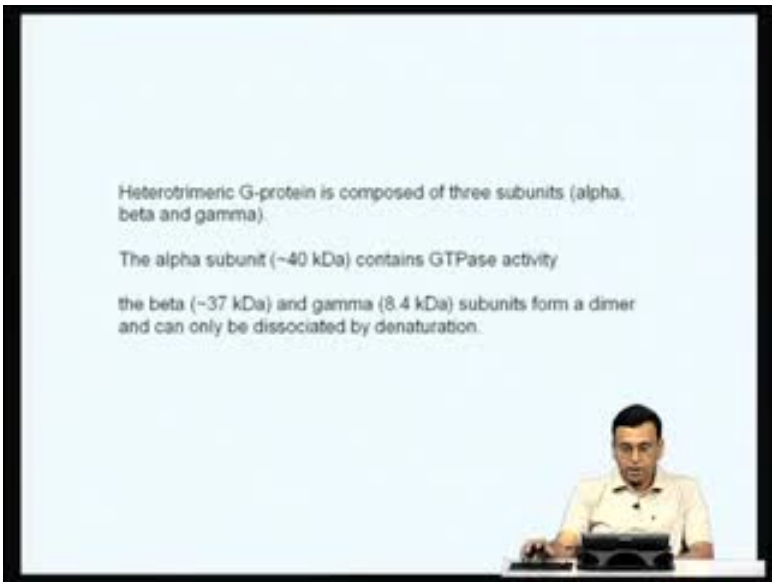

Now, the heterotrimeric G protein, which is a GTPase, is composed of 3 subunits called alpha, beta, and gamma. The alpha subunit is about 40 kilo dalton in size, and it basically contains a GTPase activity.

The beta subunit is about 37 kilo dalton in size, whereas the gamma subunit is about 8.4 kilo dalton in size, and the beta and gamma subunit together form a dimer, and is a very strong interaction. The beta and gamma subunits interact very strongly with each other. The only way we can actually dissociate with the… these 2 molecules is by actually denaturing a protein. So, it is a very strong interaction.

So, the G protein, which is the, which is the key player in the entire GPCR signalling pathway, is a trimeric molecule composed of 3 subunits– alpha, beta, and gamma, of which, the beta and gamma associate with, which are very tightly, and the alpha, which performs a number of regulatory functions.

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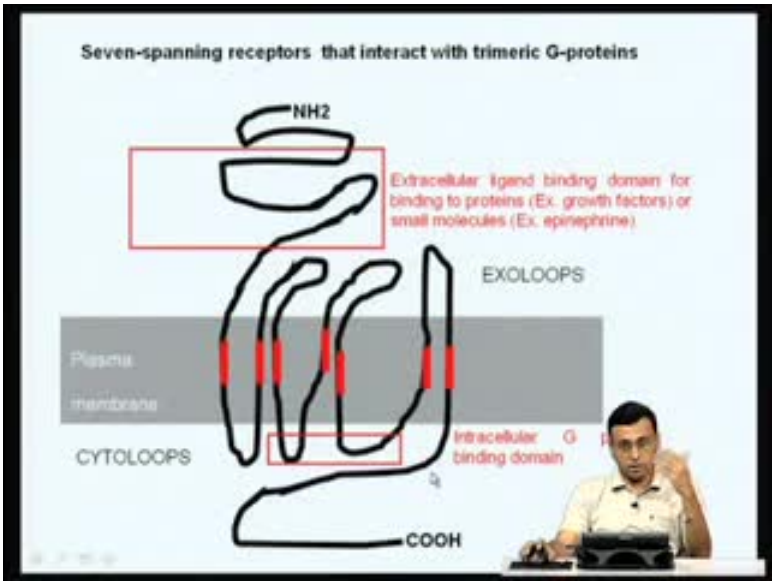

This a cartoon I have drawn, here, to actually show you the how a typical G protein or a GTPase looks, a trimeric G protein looks; trimeric G protein is activated by certain receptors, which interact with specific water soluble compounds. The activation of these G proteins is primarily carried out by the a receptor present on the cell membrane, and these receptors which activate these trimeric G proteins, contain, very characteristically, what is called as a seven-spanning membrane domains. You can see here, the ones we are shown in red here, 1, 2, 3, 4, 5, 6, 7, for our seven-transmembrane helices.

Therefore, they are often known as seven-spanning receptors, and these receptors play a very important role in trans… in translating the extracellular ligand signalling molecule

into the activation of a GTPase. We will discuss in detail how exactly this activation takes place.

So, what are the key domains of a seven-spanning receptor, which is a key player in the G protein signalling pathway. These receptors are basically transmembrane proteins, and as we can show here, there is a transmembrane domain which spans the plasma membrane, and there is an extracellular domain, and there is a domain, there is an intracellular domain which is facing the cytoplasm.

It is through this extra cellular domain that the transmembrane receptor communicates with the hormone ligand; it could be either insulin, it could be glucagon, it could be epinephrine. All these molecules interact with this extracellular domain of this transmembrane receptor, and in the intracellular domain, you have what is called as a domain that specifically interacts with the G protein of the primary GTPase, resulting in this activation.

So, these are the 2 major key domains of the receptor molecules that communicate with these hydrophilic ligands. So, often, the in the transmembrane domain, the loops which face the extracellular region are often known as exoloops, and the loops which face the cytoplasmic domain are often referred as the cytoloops. So, remember, these seven-transmembrane domains or the seven-spanning receptors play a very important role in transducing signals by certain hydrophilic molecules, which cannot enter the cell through the plasma membrane.

These contain 2 major domains– one domain is an extra cellular domain, which is involved in the communication with the growth factors of the small molecules like epinephrine, etcetera, and you also have a cytoplasmic domain, which is involved in the activation of the G protein.

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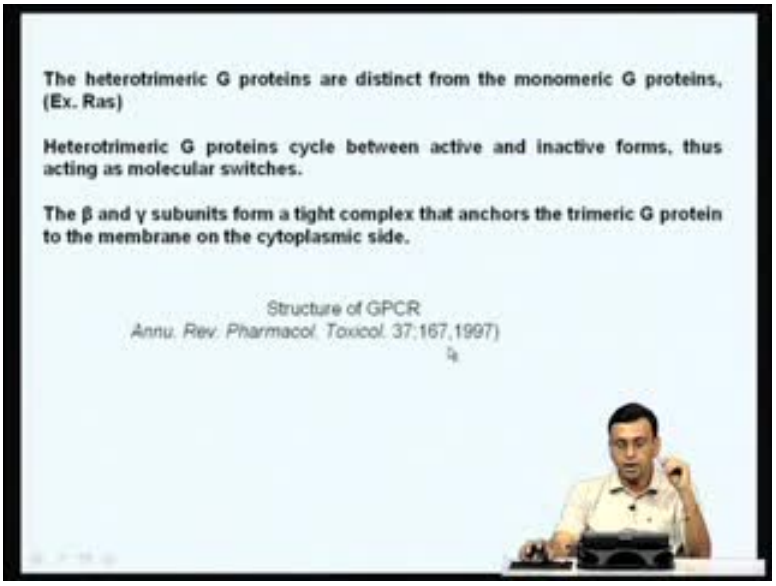

The heterotrimeric G proteins are distinct from the monomeric G proteins. Again, I do not want to confuse you people. What we will discuss in this class, today, is about these G proteins or GTPases, which are trimeric in nature. Now, as we go along, we will also now talk about another specific type of receptors, which are different from these seven-membrane transmembrane receptor we discussed so far.

These are known as receptor tyrosine kinases, which basically exists as dimers, and they activate gene expression by expressing certain growth factors like epidermal growth factor, fibroblast growth factor, and so on and so forth, and these receptor tyrosine kinases act to a different kind of a GTPase, which is a monomeric GTPase. One of the best example for monomeric GTPase is a Ras protein, and if you have mutations in this Ras, it results in cancer.

So there are 2 kinds of G proteins– one is a trimeric G protein, which are primarily activated by receptors which contain a seven-membrane domain transmembrane domain; there are monomeric GTPases, which are primarily activated by receptor tyrosine kinases which interact with a specific growth factors.

So, today's class we will discuss primarily about the trimeric G proteins, and how activation of this trimeric G proteins result in the activation or repression of specific genes. So, the heterotrimeric G proteins, which are going to discuss today, are distinct

from monomeric G proteins like Ras protein. The heterotrimeric G proteins cycle between active and inactive forms, thus acting as molecular switches.

So, the entire regulation involve in G proteins primarily involves the dynamics between the alpha beta and gamma subunits. The beta and gamma subunits form a very tight complex. As I told you, it is so tight, the only the way you can actually dissociate these two is by actually denaturation.

So, the beta and gamma subunits form a very tight complex that anchors the trimeric G proteins to the membrane of the cytoplasmic side. This a very nice review in Annual Review of Pharmacology and Toxicology about the structure of GPCR. Again, due to problems like copyright, and so on and so forth, I could not bring those crystals structure pictures here, and then actually show you, how understanding the crystal structure actually helped in understanding the various protein-protein interactions involving in the GPCR receptor. I suggest you read this review article, to actually get a very good idea about the how this structure of the GPCR receptor looks, and how it is helps in the cell-cell communication. Now, let us now try to understand how the regulation of gene expression takes place involving the G proteins.

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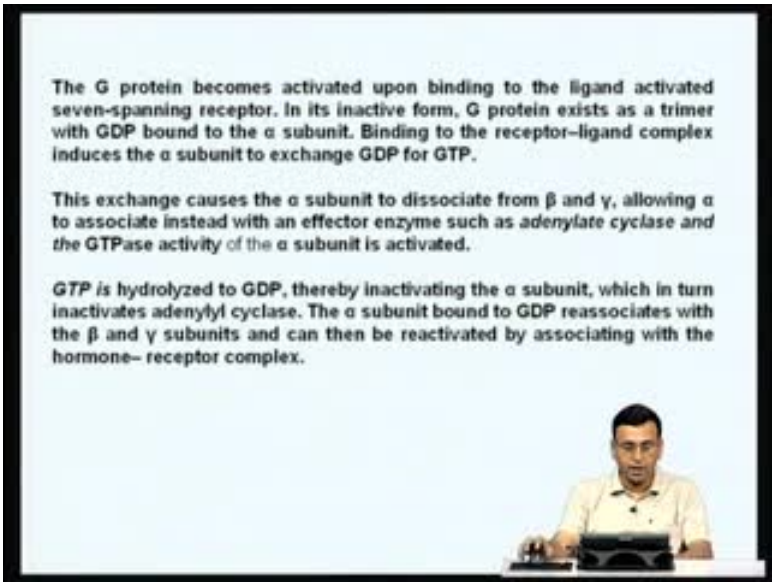

The G protein becomes activated, upon binding to the ligand activated seven-spanning receptor. So, once the ligand, the hydrophilic ligand, binds the seven-spanning membrane receptor, the seven-spanning membrane receptor now undergoes a

conformational change, and it now activates the associated GTP, and it now, which is the G proteins which is actually inactive form, exists as a trimer with alpha, beta, and gamma subunits together, and in the inactive form, the alpha subunits is actually bound to GDP.

So, remember, when the G protein is inactive, the alpha, beta, gamma, exists as a trimer, and alpha subunit remains associated to GDP. Binding of the receptor-ligand complex induces the alpha subunits to exchange GDP to GTP.

So, once the ligand binds to a seven-membrane transmembrane receptor, which induces a… It now activates a G protein, and as a result, the G protein, instead of now associating with GDP, now associates with GTP.

This exchange of GDP to GTP causes the alpha subunit to dissociate from the beta and gamma subunit, allowing the alpha subunit to associate with an effector enzyme, and these effector enzymes, usually enzymes like adenylate cyclase, and the GTPase activity of the alpha… Once the GTP bound alpha subunit is associated with adenylate cyclase, the GTP is hydrolyzed to GDP and Pi and this energy is now utilized by adenylate cyclase to is a convert ATP into cyclic AMP.

Again, the alpha subunit now becomes associated to GDP. Therefore, it becomes a dissociated, becomes inactive, and therefore, it goes and associates with alpha, beta, and gamma, and therefore, signal transduction stops.

So, the GTP is hydrolyzed to GDP, thereby inactivating the alpha subunit, which, in turn, inactivates adenylate cyclase. The alpha subunit bound to GDP re-associate the beta and gamma subunits, and it can again be reactivated by another molecule of ligand binding receptor, and again the entire cyclic continues.

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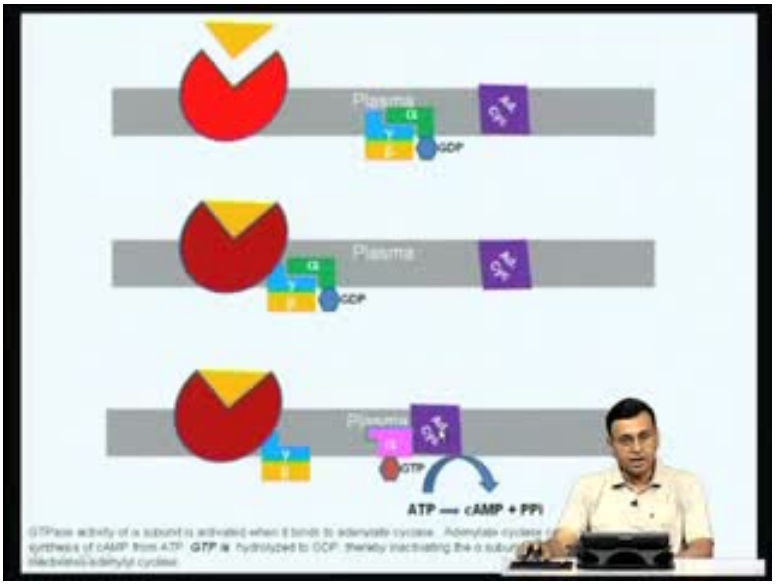

So, you can see, the mechanism is very simple. I will explain this whole thing with the cartoon here. This is what I shown, here is a hydrophilic ligand, which could be either molecules like insulin receptor, or could be small molecules like epinephrine, and here is the trans seven-membrane receptor, which is present in the plasma membrane. And we have the GTPase, which is shown here, the alpha, beta, and gamma subunits in 3 different colors, and inactive form of the G protein is bound to the GDP here, and adenylate cyclase somewhere nearby.

This, here there is no signal transduction, and let us see, the moment, now, this extra cellular molecule binds to seven-membrane receptor, the alpha, beta, and gamma subunit of the G protein the seven-membrane receptor undergoes a conformational change, and as a result of it, the trimeric GTP dissociates, so that the only the gamma, beta subunit now associate with the receptor, whereas the alpha subunit dissociates from the trimer and it, in GTP bound form, it now activates adenylate cyclase, associate adenylate cyclase and activates, and therefore, the energy you release by the GTP hydrolysis is now used for converting for the production of cyclic AMP from the ATP by adenylate cyclase.

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Once this is done, again, the one the GDP and all is over, now the GDP-bound alpha subunit again becomes inactive, and it go back and re-associate with gamma and beta subunit, and adenylate cyclase now becomes inactive. So, this is how the binding of a hormone to a cell surface receptor results in synthesis of cyclic AMP involving the trimeric G protein. So, following GTP hydrolysis, the GDP-bound alpha subunit of G protein re-associates with the heterotrimeric G protein, and is ready to be reactivated by a second hormonal stimulus.

So, hope this cartoon has now explained the entire event that takes place. Once a signalling molecules bind to a seven-membrane receptor present on the cell surface, the receptor undergoes a conformational change. As a result, the trimeric G protein, now, the only the beta and gamma subunit remain associated with the receptor; the alpha subunit dissociates, the alpha subunit now goes and interacts the adenylate cyclase. GTP is hydrolyzed, cyclic AMP is synthesized, and again, the GDP bound form of the alpha subunit now again becomes inactive, comes back, re-associate the beta and gamma subunits, and adenylate cyclase is inactivated.

So, a burst of cyclic AMP is synthesized the moment a ligand binds to receptor. The entire event takes place and 1 molecule interact, 1 molecule of hormone interacting with the receptor results in the synthesis of hundreds and thousands molecules of synthesis of cyclic AMP during this very brief period.

Here is a, now again, at least 2 articles which discuss, in great detail, about this entire mechanism of cyclic AMP synthesis involving hormone receptor interactions.

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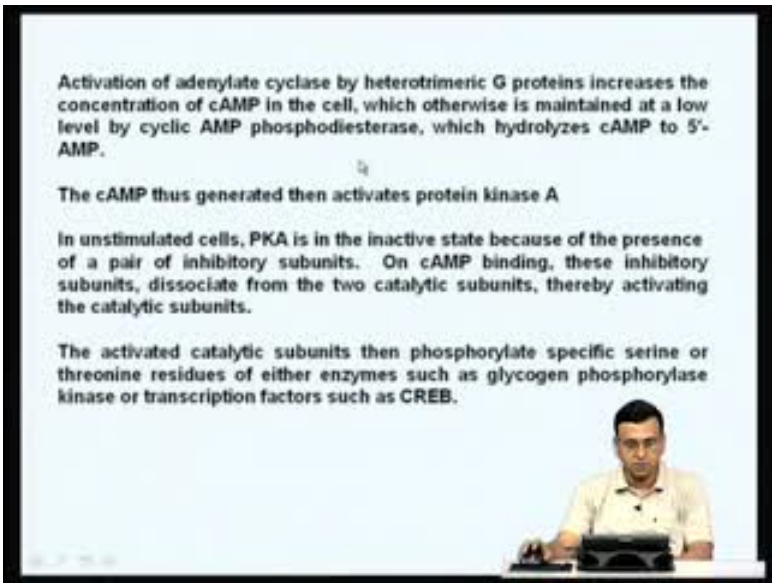

So activation of adenylate cyclase by heterotrimeric G proteins increases the concentration of cyclic AMP in the cell, which, otherwise, is maintained at a very low level by cyclic AMP phosphodiesterase, which hydrolyzes cyclic AMP to five prime cyclic AMP.

So, you can see, these are all very important regulatory molecules, and, so, all these things have a very transient half-life. They are made; and they are immediately degraded. So, when there is no hormonal stimulus, the cyclic AMP levels are actually very low inside the cell, because as soon as the cyclic AMP is made, it is immediately cleaved by enzymes like phosphodiesterase, cyclic AMP phosphodiesterase, which immediately cleaves the cyclic AMP.

So, these second messengers, because of their very powerful regulatory activity, their half-life is very short, and is very highly governed by the degrading enzymes. So, the cyclic AMP thus generated by the activation of adenylate cyclase, now the cyclic AMP goes and activates protein kinase A. In the unstimulated cells, protein kinase A is exists as a inactive form, because of the presence of a pair of regulatory subunits.

So I have what are called as R2 C2, again, we discuss this structure of protein kinases in regulation, when you discuss about the in the previous classes, when you discuss the mechanism by which the cyclic AMP response element binding protein activates gene expression.

Now, there we actually shown using a cartoon, how the protein kinases exists in a inactive form. We have R2 C2, as well as the regulatory subunit and catalytic subunits are together. It is in active form, but the moment the cyclic AMP levels inside the cells goes up, cyclic AMP now goes and binds a regulatory subunit of a protein kinase A. Now, as a result, the regulatory subunit dissociates and the catalytic subunit is now free. Now, it becomes active. Now, it goes and either phosphorylases and specific enzymes involved in a metabolic pathway, or it will go and phosphorylate, go to the nucleus and phosphorylate transcription factors, such as the cyclic AMP response element binding protein or CREB.

So, in unstimulated cells, the PKA is in inactive state because of the presence of a pair of inhibitory subunit or regulatory subunits. On cyclic AMP binding to these regulatory inhibitory subunits, they dissociate from the catalytic subunit, thereby activating the catalytic subunit.

The activated catalytic subunits, now, go and phosphorylate specific serine residues or threonine residues of enzymes such as glycogen phosphorylase kinase, or they can go and phosphorylate transcription factors such as CREB.

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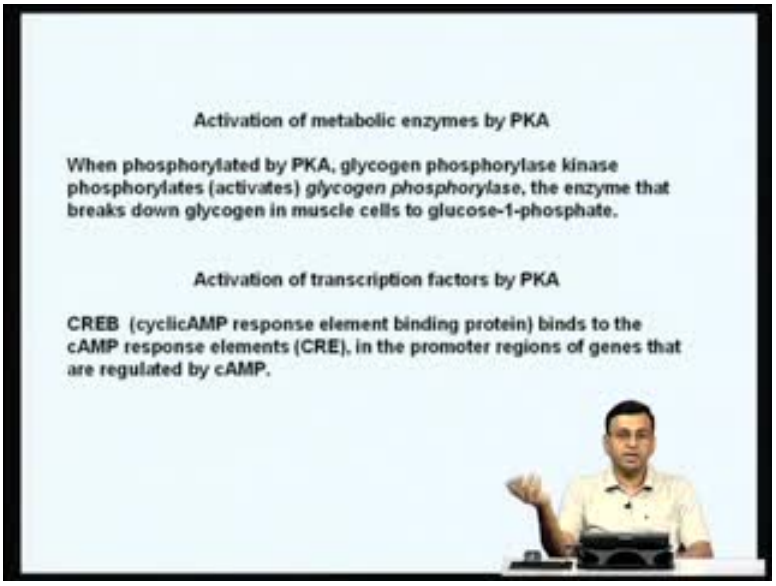

Now, on to the consequence of this– as a consequence of this, when there is an increase in the cyclic AMP levels inside the cells, and when there is an activation of protein kinase A inside the cells, it can either result in the activation or metabolic enzymes, or it can result in the activation of transcription factor. That is what I shown here. The PKA, on phosphorylation, when phosphorylated by PKA, the glycogen phosphorylase kinase phosphorylates or activates another enzyme called glycogen phosphorylase, and this enzyme, now, involved in the breakdown of glycogen in muscle cells to glucose-1-phosphate.

For example, this is what happens during glucose metabolism. When glucagon is produced, the glucagon binds to this corresponding glucagon receptor, which is a seven membrane receptor, and this results in cyclic AMP. Cyclic AMP now comes and activates protein kinase A; protein kinase A now goes and phosphorylates the glycogen phosphorylase kinase. Now, it becomes active, glycogen phosphorylase kinase now goes and activates glycogen phosphorylase; that goes and now breaks down. That is now involved in the breakdown of glycogen, leading to the production of glucose-1-phosphate in muscles.

So, this is how our body responds whenever there is elevated levels of glucagon. So, when there is no glucose in the cells in the… in the circulation in the blood, the glucagon is released by pancreas, and glucagon comes and binds the seven-membrane receptor,

and that now phosphorylates, leads to synthesis of cyclic AMP, leading to the activation of glycogen phosphorylase, ultimately increasing the blood concentration of glucose.

Now, the same protein kinase A, in addition to activating the enzymes involved in the glucose metabolism or glycogen metabolism, can also now go inside the nucleus and phosphorylate the cyclic AMP response element binding protein, which now goes, and which, now, can interact with the histone acetyltransferases, HAT, especially this CREB binding protein or the CBP, also known as the p300, and as a result, there is a faster recruitment of transcription factor, leading to activation of gene expression.

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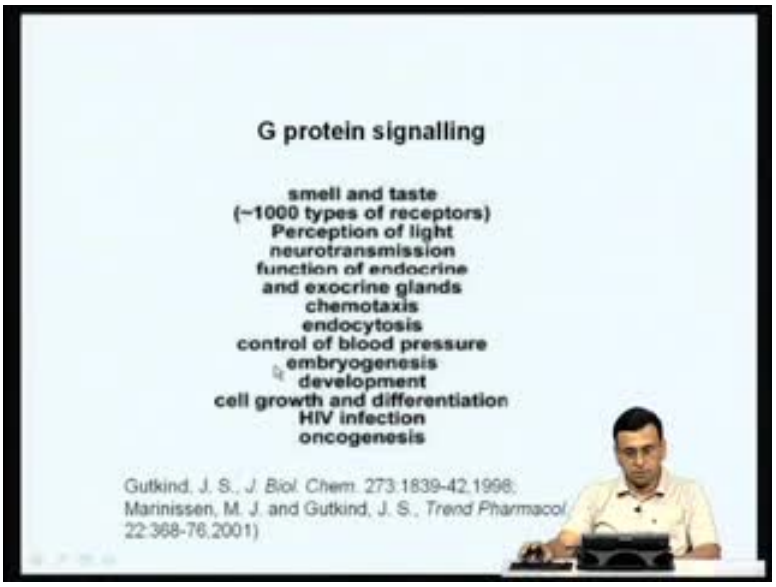

So, you can see, how interaction of these hydrophilic or the lipid insoluble molecules like glucagon, or epinephrine, or even insulin, this and so on and so forth molecules, by interacting with specific membrane receptors, can result in the activation of a GTPase, result in the synthesis of cyclic AMP. Cyclic AMP now activate protein kinase A, and this protein kinase A either can activate metabolic enzymes, or it can activate transcription factor, leading to activation or repression of genes.

Now, this is these kind of a G protein-involved signalling is used by a number of… is used a number of physiological responses. I have listed some of this things here. Smell and taste involves G protein signalling; there are more than 1000 types of this kind of a seven-membrane transmembrane receptors, which are involved in sensing our smell and

taste. The way we pursue smell, the way we taste things, all these things involved G protein signalling.

Perception of light involves the seven membrane receptor. The rhodopsin, one of the major receptors involved in perception of light, is a seven-membrane transmembrane protein. Neurotransmission, function of exocrine and endocrine glands, chemotaxis, endocytosis, control of blood pressure by epinephrine, non-epinephrine, so on and so forth, embryogenesis, development, cell growth and differentiation, HIV infections, oncogenesis, you can see a number of physiological processes make use of this kind of G a protein signalling, involving a trimeric G protein receptors, ultimately leading to the intracellular increase in certain messengers like cyclic AMP, leading to activation of specific G protein signalling pathways, and a number of nice reviews I have listed here, which you can go through it and understand, how G protein signalling plays a very important role in the activation of a number of in the development number of a physiological responses.

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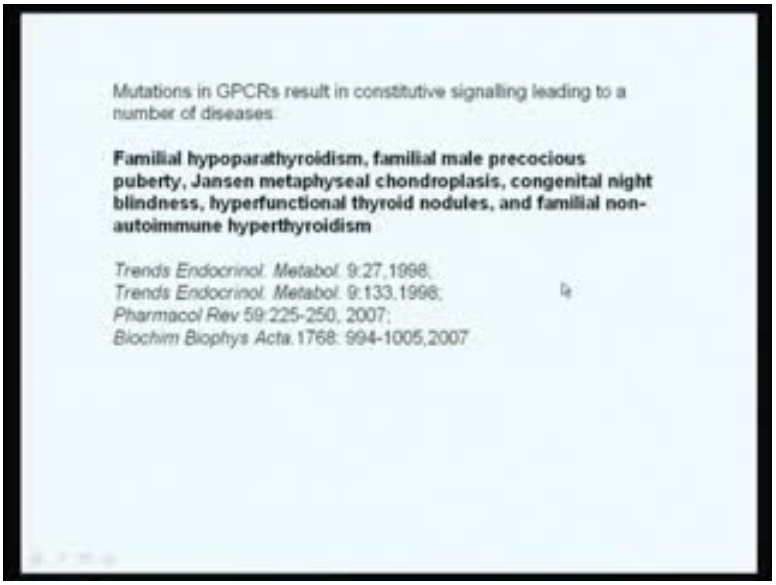

Mutations in the GPCRs, the G protein coupled receptors, result in constitutive signalling. If you have mutations in the G protein subunits, then the alpha protein becomes, the alpha subunits becomes the constitutively active, and as a result, there is no regulation; the GTPases is constitutively active. Therefore, it constitutively interacts with the adenylate cyclase, the cells continuously producing cyclic epinephrine. As a result,

there is a continuous activation of the enzymes of the metabolic pathways, or continuous activation of transcription factor, and that can have disastrous consequences.

When these kinds of things happen, it results a number of diseases, some of which I have mentioned here, like familial hyperthyroidism, familial male precocious puberty, Jansen metaphyseal chondroplasis, congenital night blindness, hyper functional thyroid nodules, familial non autoinmune hyperthyroidism, and so on and so forth. There are nice reviews I have listed here, which discussed how defects in G protein signalling can lead to a number of diseases.

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I have also given a table, here, which gives a much more detail about how defects in some of the G protein coupled receptors can lead to a number of diseases; what kind of point mutations have been shown to be involved in these G protein coupled receptors, and what kind of diseases it manifests in.

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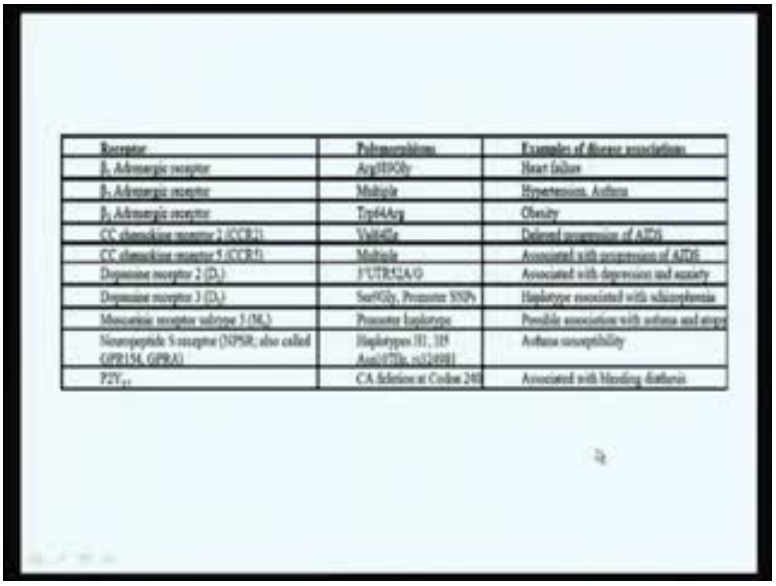

There is continues, like adrenergic receptors, dopamine receptors, all these have G protein coupled receptors, and when you have mutations in these things, it results in the manifests of a, or manifests in the form of a specific disease.

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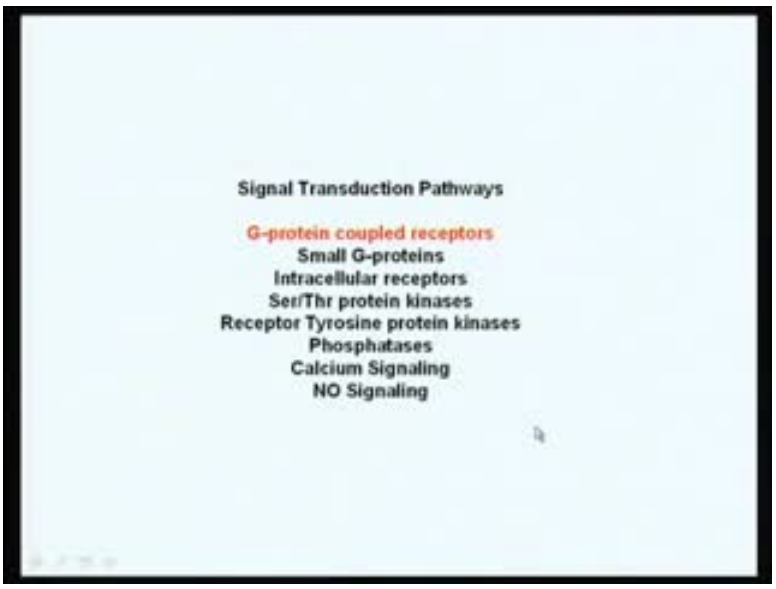

So, what we discussed today is about 1 major signal transduction pathway, which involves interaction of certain water soluble molecules with G protein coupled receptors or GPCRs, which are seven membrane, which contain a seven-membrane transmembrane domain, and this interaction activates a GTPase, which is a trimeric

protein. And the trimeric protein– this is this the activation involves dissociation of the alpha subunit from the beta gamma subunits. This alpha subunits now goes and activates adenylate cyclase that results in the synthesis of cyclic AMP. Cyclic AMP now come and go, now goes and activates protein kinase A; that, ultimately, now either activates enzymes of metabolic pathways, or activate genes of a particular transcription pathway.

There are many other signal transduction pathways which involves smaller G proteins such as Ras, there are intracellular receptors, there are serine and threonine protein kinases which are involved in signal transduction pathways, there are receptor tyrosine kinases, and there are phosphatases, which negate the action of kinases; there is calcium signalling, nitric oxide signalling. What we will do, in the next few classes, is to take all, or at least some of these as examples, to understand how activation of each one of the signal transduction pathways, ultimately results in the activation or repression of specific gene expression programs.

I think I will stop here. So, what I have discussed in this class today, is to introduce you to the area of signal transduction, and I the message I have conveyed to you is that activation or repression of transcription of genes inside the nucleus is not just confined to nucleus alone. This whole thing happens in response to a variety of stimuli that the cell encounters during its life.

So, depending upon whether the signalling molecules are capable of entering the cell and interacting with specific intracellular receptors, or whether they interact with specific membrane receptors, when they have both this mechanisms, ultimately results in the generation of the second messenger molecules, or it involves the activation of specific phosphorylation cascades, and as a result, specific transcription factors are activated, ultimately resulting in activation or repression of specific genes inside the nucleus. And 1 signal transduction pathway which we discussed in detail today involves the G protein coupled receptors, through which trimeric G proteins are activated, and this trimeric G proteins, in return, activates enzyme like adenylate cyclase, results in the synthesis of cyclic AMP, which then phosphorylate, which then activate protein kinase A, which, in turn, activates specific gene expression programs.

So the coming (( )), will take up a few more examples of the signal transduction pathways, and understand how molecules, interacting either in cell surface receptors or

with the intracellular receptors, ultimately activate the or repress transcription of specific genes. Thank you.