Eukaryotic Gene Expression: Basics and Benefits

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Lecture No. # 20
Regulation of gene expression by cytokines

Let us continue our discussion about regulation of gene expression by molecules which binds inter cell surface receptors. Binding of these molecules for cell surface receptors ultimately through specific signal transaction pathways lead to the activation of specific genes which ultimately manifest into distinct physiological responses. Now, in the last 5 classes we have not discussed about these kinds of signal transaction pathways.

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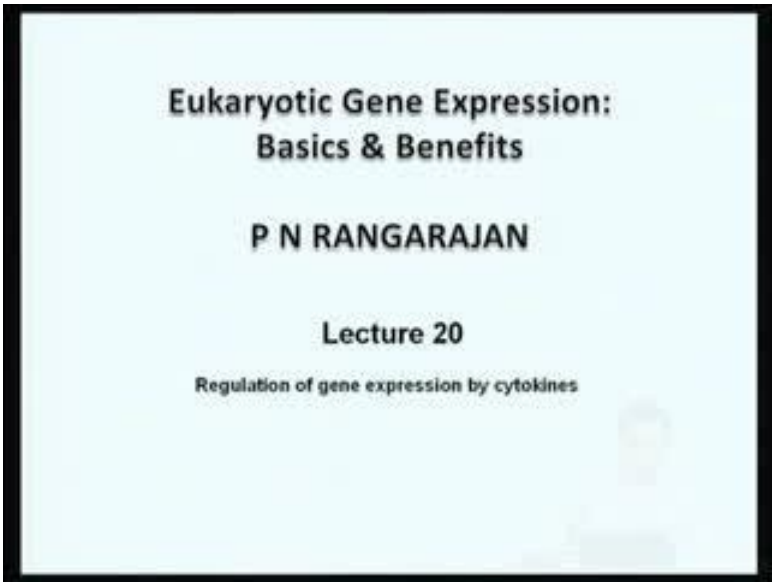

I am trying to give you overall picture of how these signalling molecules which interact with specific membrane receptors can transduce signals through various signal transaction pathways involving specific protein kinase and ultimately leading to the activation of specific transcription factors and activation of specific target genes. So, just to recapitulate what we have. So, today what will discuss is how these kinds of a regulation of gene expression takes place by a distinct group of molecule called cytokines. So, this will be the today's topic.

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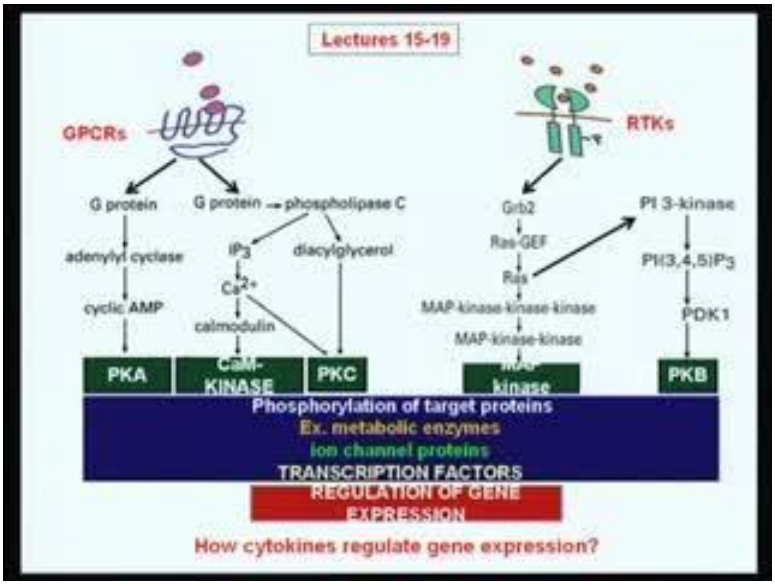

In the previous two topics, we have discussed about signalling molecules which act with transcription through G protein couple receptors. We also discussed molecules which like growth factors which bind to growth factor receptors and then activate transcription through the Ras pathway leading to activation of specific genes. So, just to recapitulate what we have been discussing just summarized here the almost what we have been discussing in the last 4 lectures.

We began by discussing a group of molecules signalling molecules which interact with specific membrane receptors known as GPCRs or the G protein couple receptors which are 7 transmembrane proteins and when these signalling molecules interact with these GPCRs, it results in the activation of G proteins. We have discussed about what kinds of G proteins are, they are primary G proteins which consist of alpha, beta and gamma sub units and when the molecule interacts with the receptor GPCR, it alters the confirmation of the receptor and resulting in the activation of the G protein.

The alpha sub unit of G protein now dissociates and this alpha G sub unit and active GTPs now activates adenylyl cyclise. Adenylyl cyclase then synthesizes cyclic AMP which is very important second messenger. Cyclic AMP interacts with protein kinase a.

We also discussed, how the same in certain cases when certain signal molecules drop with a GPCRs. It can also lead to different kind of G proteins and these kinds of G proteins instead of activating adenylyl cyclase, they can actually activate phospholipase C and this activated

phospholipase C now converts the 1 3 bis-inostal diphosphate into inostal triphosphate and diacylglycerol. Diacylglycerol then goes and activates protein kinase C whereas the inostal triphosphate goes to enter process in criticulum releases calcium. Calcium has two things. It can either go or activate calmodulin and this in turn can activate calmodulin kinases or the calmodulin calcium can also go along with diacylglycerol can activate protein kinase c.

So, this is what we discussed about the GPCRs. We also discussed in detail how certain growth factors poly peptide growth factor, ilex epidermal growth factor, fibroblasts growth factor, platelet derived growth factors etcetera. How, when they interact with specific membrane receptors known as receptor tyrosine kinases?

The binding of the sibling molecules with these receptors activates the tyrosine kinase activity of this receptor and these two monomers cross phosphorylate each other from the tyrosine residues and these tyrosine phosphorylated receptors now interact with specific adaptor proteins like the Grb2 which then activates the one GTA (( )) exchange factor and then which activates the Ras.

Ras is a monomeric GTPS which is very similar to the g alpha subunit of the primary G protein and this activated Ras and then activates what is known as the map kinase signalling pathway involving map kinase-kinase-kinase which then activates map kinase-kinase and it activates map kinase.

There are also certain things which we did not discuss because it is not possible to cover all the signal transaction pathways. The same Ras which has been activated by the binary growth factors can also go and activate a PI3 kinase phosphotolinus pol 3kinase. This ultimately leads to different kind of signalling pathway and this can lead to the activation of protein kinase B. So, what we have discussed so far is that different molecules instructing through distinct signal different signalling pathways can lead to the activation of different protein kinases.

In one case, protein kinase A is activated. In other case, calmodulin kinase is activated or protein kinase C map kinases or protein kinase B. Now, what is the purpose of all these things? Ultimately, all these kinases go and phosphorylate number of target proteins which varies from cell type to cell type depending upon the physiological situation as well as cross talk with other signalling pathways and these target proteins can be either metabolic enzymes, it could be ion channel proteins or it would be transcription factors.

In these lectures series, we focused primarily attention of how all these signalling pathways lead to the activation of transcriptional factors and once these transcriptional factors are activated, it ultimately results in the activation or repression of specific target genes which in turn manifest into distinct physiological responses. So, this is what we discussed in the last 5 classes.

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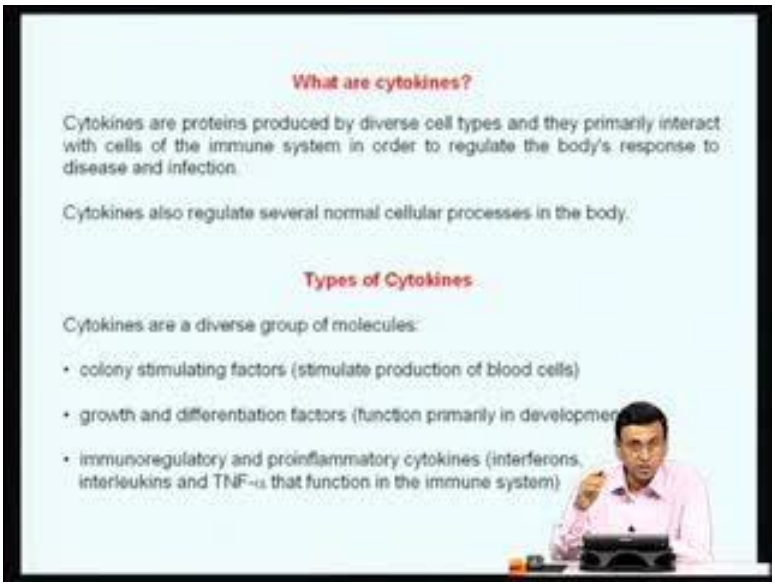

Today, we will talk about another important group of molecules other than those the G protein ligands as well as the receptor kinases. Let us now talk about a new set of molecules called cytokines. How cytokines regulate gene expression? This is what we are going to discuss today. Now, cytokines are proteins produced by diverse cell types and they primarily interact with cells of immune system in order to regulate the body's response to disease and infection.

So, the cytokines play a very important role in the way, we fight various infections whether it is viral infections, bacterial infections, parasite infections. The cytokines play very important role in modulating our immune functions. The cytokines also regulate several normal cellular processes in the body. So, they are very important biological molecules. Now, what are the various types of cytokines? Cytokines are very diverse group of molecules; they include colony stimulating factors, many of these like GCSF, GMCSF etcetera. They stimulate the production of blood cells such as red blood cells, platelets and so on so forth. There are also cytokines also involves growth and differentiation factors with function primarily during

development. When we study the importance of regulation of gene expression during development, we will come back and revisit some of these molecules.

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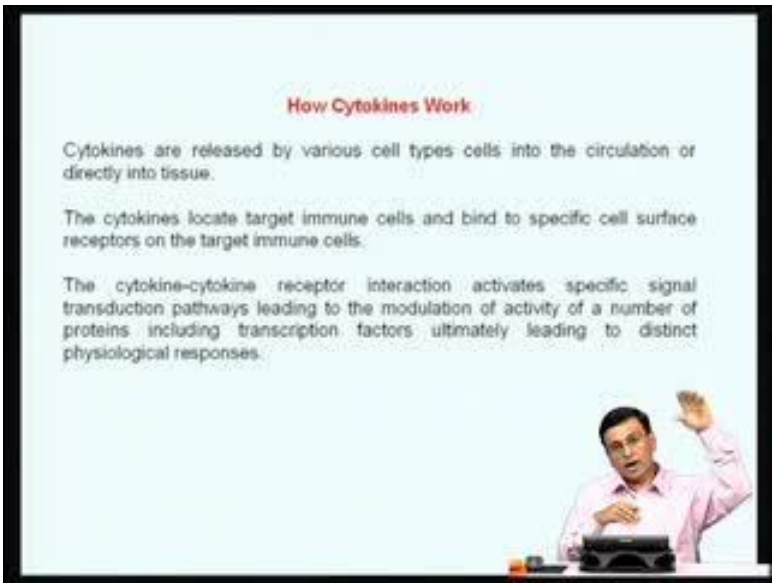

Cytokines also are immuno-regulatory and pro-inflammatory cytokines and these include very important molecules such as interferons, interleukins and tumour necrosis factor alpha. All these molecules play very important role in regulating the function of the immune system, especially the T cells, B cells and so on and so forth.

How do these cytokines work? Cytokines are actually released by various cell types into the circulation or sometimes directly into a particular tissue and once these cytokines are released, these cytokines then go and interact with specific target cells. This interaction primarily takes place through specific cell surface receptors which are present on the target immune cells. So, there are cells which produce these cytokines. These are released into the blood stream and these cytokines then go and bind to the cell surface receptors of specific target cells. This cytokine receptor interaction then activates specific signal transaction pathways leading to the modulation of activity of a number of proteins which also includes transcription factors. Ultimately, all these things manifest in the form of distinct physiological response.

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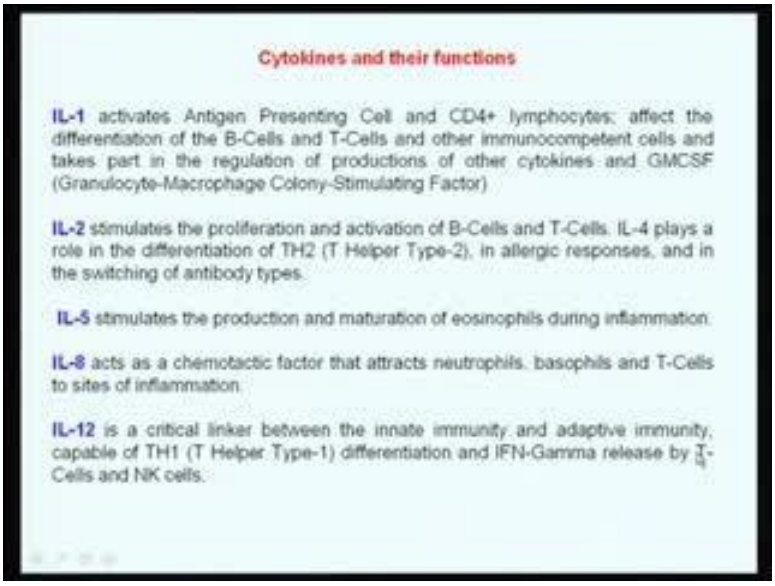

So, this is the molecules like interferons, interleukins, erythropoietin, growth for growth hormones. All these molecules primarily act through this particular signalling pathway. This is the signalling pathway we have also discussed in the previous lectures is the same mechanism by which other signalling molecules also activate expression of specific target genes. Let us now discuss, what is the distinction between the way the cytokines activates specific signal transaction pathways and molecules like growth factors and other G protein couple receptor mediator pathways are different.

Now, before we discuss the mechanism by which the signal transaction pathways take place, let us now briefly discuss what are the various kinds of cytokines and what are their importance. The reason we need to understand is because unless the importance of the cytokines, there is no point in discussing about these various signal transaction pathways. So, what I have listed in the less couple of slides is to variously introduce what are then very important cytokines. What are the normal functions and then it becomes very clear why we need to understand the mechanism by which these cytokines work.

For example, there are whole bunch of molecules known as interleukins which for very important group of cytokines and there are very diverse type of interleukins. I will list out some of these and also discuss the functions of some of these cytokines. For example, we have interleukin 1. Interleukin 1 primarily activates antigen presenting cells as well as CD4 positive lymphocytes after the differentiation of B cells and T cells. They also affect the

differentiation of B cells and T cells as well as many immuno-competent cells and they also take part in the regulation production of various other cytokines like the granulocyte macrophage colony stimulating factors or GMCF.

Remember, molecules like interleukins and genes are very important biological molecules, have lot of biomedical importance in treatment of variety of diseases. Similarly, interleukin too stimulates the proliferation and activation of B cells and T cells and it plays a very important role in the differentiation of T helper type Th2, T helper 2, T cells as well as in allergic responses and switching of various antibody types. If we now take the interleukin 5, interleukin 5 primarily stimulates the production and maturation of eosinophil during inflammative responses.

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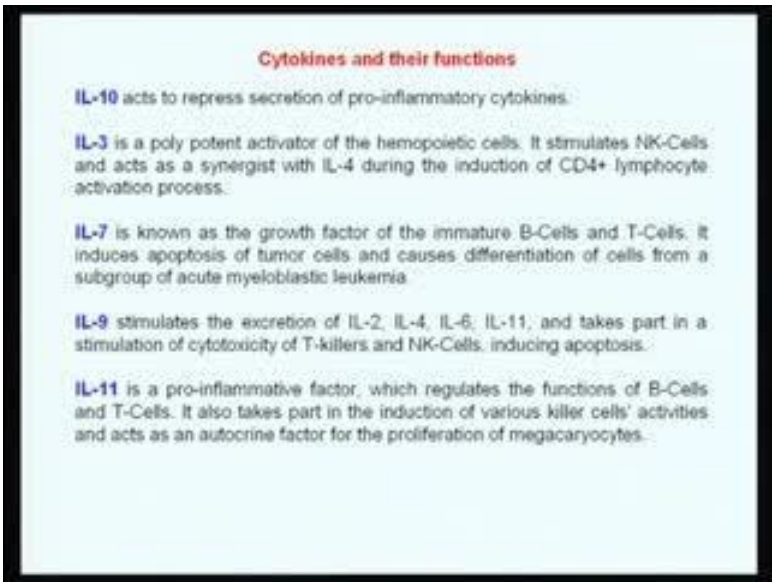

Whereas interleukin 8 acts as a chemo tactic factor that attracts neutrophils, basophiles and T cells to the sites of inflammation. We can see these molecules are very important in many of these processes. Similarly, interleukin 12 is a very critical linker between innate immunity and adaptive immunity capable of Th1 differentiation and interferon gamma release by T cells as well as natural killer cells. So, all these interleukins play very important role and regulate a number of important biological responses. Similarly, if we look at interleukin 10, then interleukin 10 acts as repress secretion of pro-inflammatory cytokines. So, it suppresses inflammation is an anti-inflammatory factor. Interleukin 3 is a potent activator of

hematopoietic cells. It stimulates natural killer cells and also acts as synergy with IL 4 during the induction of CD4 positive lymphocyte activation process.

Interleukin 7 is known as a growth factor of the immature B cells and T cells. It induces apoptosis of tumour cells and causes differentiation of cells from a subgroup of active myeloblastic leukemia, a very important and difficult form of cancer. Interleukin 9 stimulates the excretion of IL 2, IL 4, IL 6 and IL 11 and takes part in a stimulation of cytotoxicity of T cells, T killer cells and natural killer cells inducing apoptosis whereas interleukin 11 is a pro inflammative factor. It regulates the functions of B cells as well as T cells and it also takes part in the induction of various killer cells activities and acts as an auto-prime factor for the proliferation of megakaryocytes. So, you can see these diverse groups of interleukin play very important role in a number of biological processes. So, it is very important for us to understand how do these molecules are able to bring out these distinct physiological responses.

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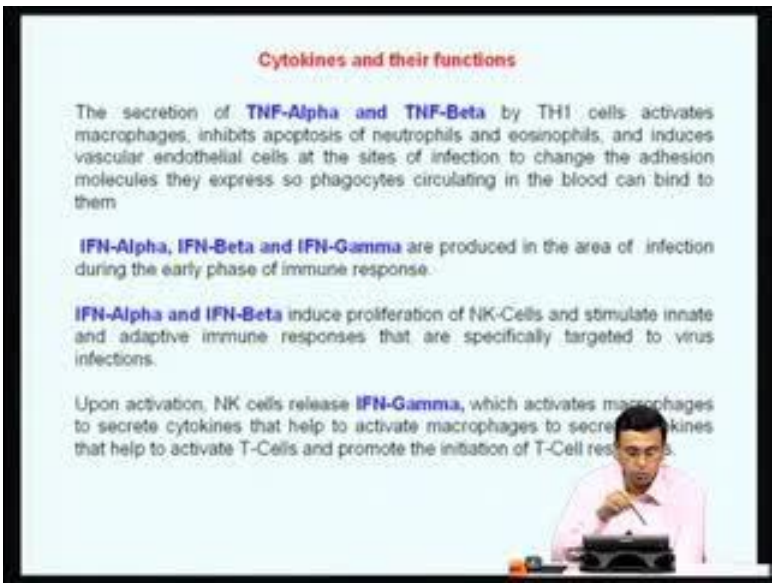

Similarly, if we now take another group of cytokines like TNF alpha and TNF beta, the secretion of these TNF alpha and TNF beta, TNF stands for tumour necrosis factor. There are two types, TNF alpha and TNF beta. These are secreted by the Th1 helper cells and they activate macrophages. They inhibit apoptosis of neutrophils and eosinophils and induce vascular endothelial cells at the sites of infection to change the adhesion molecules. They express so that phagocytes circulating in the blood can bind to them.

So, has a very important role in immuno-surveillance during infection as well as disease processes. Similarly, interferon's which consists of interferon alpha, interferon beta, interferon gamma; they are produced in the area of infection during very early stages of immune response. Those who study immunology understand that these molecules are very important for the immune functions. Interferon alpha, interferon beta for example they induce proliferation of natural killer cells and stimulate innate as well as adaptive immune responses and they are specifically targeted for virus infections.

So, the interferon's play very important role in a ability of our body to control various viral infections. Upon activation, the natural killer cells actually release interferon gamma which activate macrophages to secrete various cytokines and these cytokines in turn activate macrophages to secrete cytokines that help to activate T cells and promote the initiation of specific antigen specific T cell responses.

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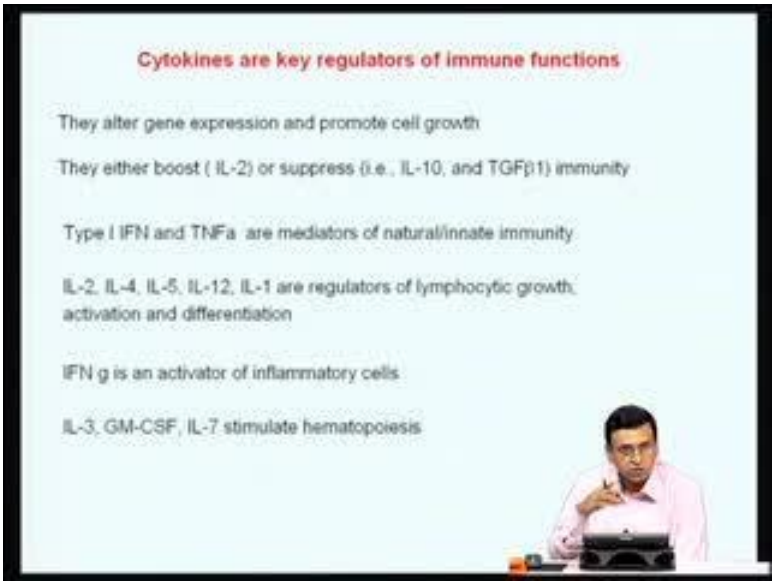

So, you can see these molecules play very important in both humoral as well as cell meditative immune responses. So, to put in a nut shell, the cytokines are key regulators for various immune functions. For example, they alter gene expression. This is what we are going to study. How these cytokines by interacting with cell surface receptors how they alter gene expression is what is going to be the focus of today's talk. How do they alter gene expression? How do they promote growth? They either boost interleukin 2; they sorry these cytokines either boost or suppress immune responses. For example, if we take molecules like

interleukin 2, they actually boost immune responses whereas molecules like IL 10 and TGF beta 1, suppress the immune responses.

So, they are both positive as well as negative regulators of immune responses whereas type 1 interferons and type A tumour necrosis factor alpha are mediators of natural as well as innate immunity whereas the various interleukins listed here, they are regulators of lymphocyte growth activation differentiation. Interferon gamma is an activator of inflammatory cells whereas interleukin 3 GM CSF of an IL 7 stimulate hematopoiesis like production of red blood cells, platelets and so on and so forth.

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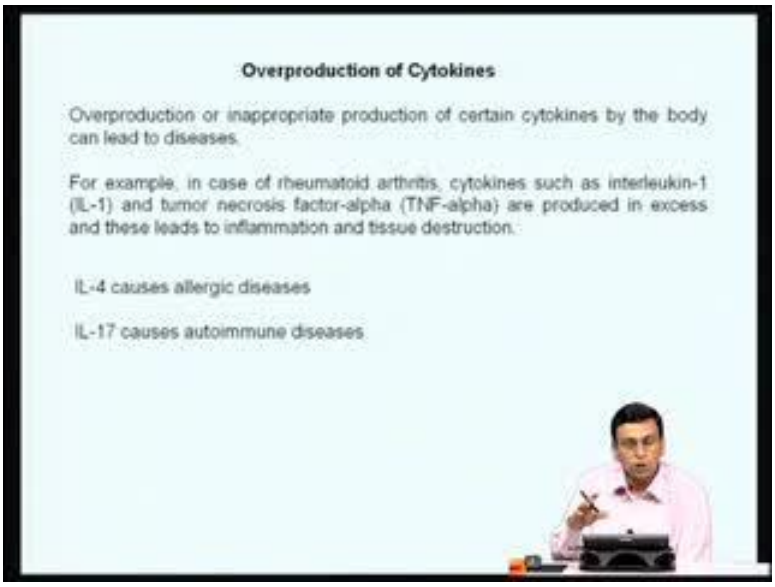

So, I spent all this time to primarily bring to your attention that the molecules or the signalling transaction pathways, we are going to discuss are very important and these molecules play very important role in number of immune functions. So, we need to understand how do these molecules act and how do they transduce signals once they bind to distinct cell surface receptors.

The other important reason why we have to know how this molecules act is that when these cytokines which are discussed so far, when theses cytokines are over produces, over producing the body or when they are not produced properly or when the production is not properly regulated, it can lead to number of disease processes. For example, over production of or inappropriate production of certain cytokines, it can lead to number of diseases. The example is in the case of rheumatoid arthritis. For example, cytokines such as interleukin 1 as

well as tumour necrosis factor alpha are produced in excess and when these cytokines are produced in excess, it leads to rheumatoid arthritis, inflammation of joints and so on and so forth.

So, a number of companies have either drop out or in the process of developing molecules which can suppress the production of these interleukin, so that you can reduce inflammation and you can fire a cure for rheumatoid arthritis. Similarly, interleukin 4 when it is produced, how much it causes involved in allergy and allergic diseases whereas interleukin 17 is involved in a number of auto-immune disorders.

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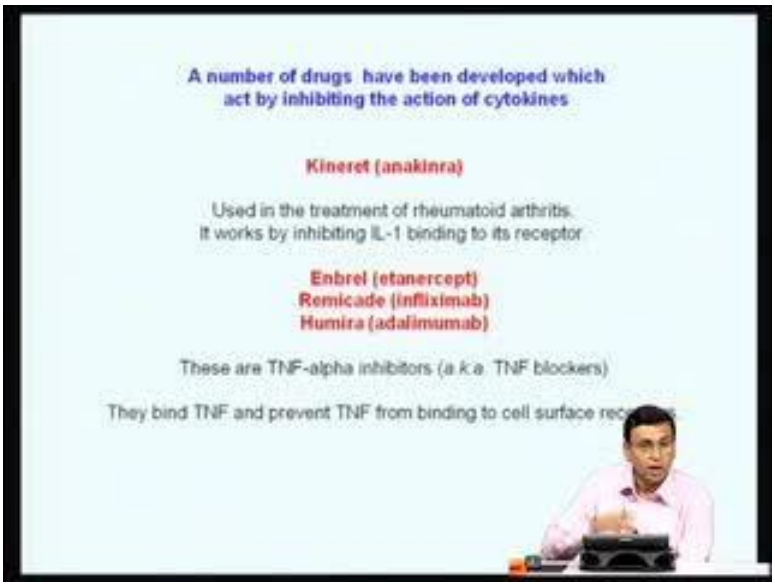

This is just a few examples to tell you many of these molecules are very important and if they are not properly produced or if their regulation is not proper, it can manifest in the form of specific disease processes. So, in view of the importance of these molecules in various biological processes as well as its importance in controlling these inter-signalling molecules, a number of drug companies have invested lot of money to understand the mechanism while these cytokines act and in the process have discovered number of drugs which either activate or act as inhibitors of these various cytokines and many of these drugs are already in the market.

So, a number of drugs have been developed which act by inhibiting the action of cytokines. I just listed a few examples here and towards the end of this lecture, we will again revisit this and look at various other drugs which have been developed for various diseases. For

example, we have a drug called kineret also known as anakinra which is as used in the treatment of rheumatoid arthritis. It basically works by inhibiting interleukin 1 binding to the receptor. So, it is antagonise for interleukin 1. When it goes and binds to the interleukin receptor, it prevents binding of the interleukin 1 and as a result, the entire signal path transaction pathway leading to gene expression is blocked and this has a very important effect. So, these kinds of inflammatory cytokines the effect, the inflammation are now reduced. Therefore, it is very widely used for rheumatoid arthritis.

Similarly, molecules like Enbrel, Remicade, Humira they are all very important drugs. They are all TNF alpha inhibitors, popularly known as TNF blockers. So, they all block the TNF mediator signal transaction pathway. They basically bind TNF and prevent TNF from binding to the cell surface receptors. So, you can see by blocking these molecules from interacting with the cell surface receptor, you can develop a number of therapeutic agents for specific diseases whether it can be rheumatoid arthritis, inflammation or a number of other disease processes.

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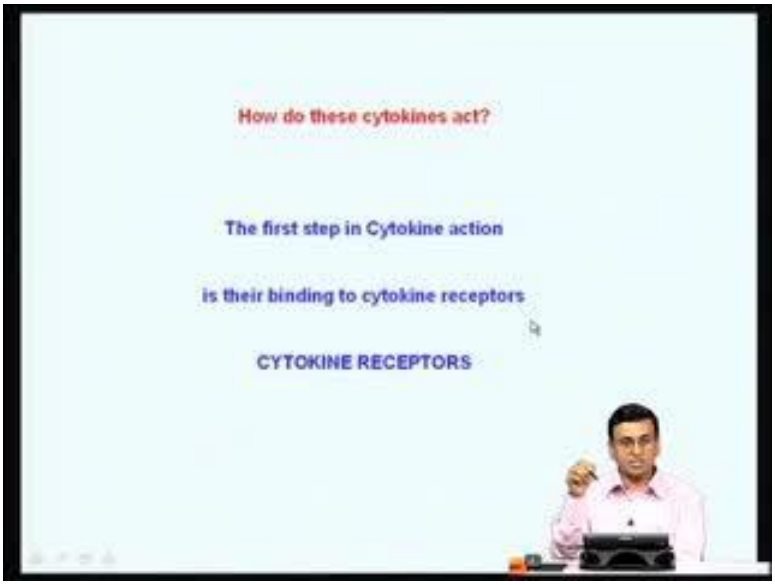

So, it is very important for us to understand what happens in the normal process and what kind of signal transaction pathways are blocked, when you block the interaction of these molecules with the cell surface receptors. So, having discussed so far the importance of these cytokines, how they control a number of important biological processes right from production of erythropoesis or various blood cell types as well as immune system functions.

Now, let us try to understand how do these cytokines are able to bring out these distinct physiological responses. So, the first step in the cytokine action is their binding to cytokine receptors. So, this is how this is the first step in the cell cytokine signal transaction pathway. So, let us now try to understand what kind of cytokine receptors interact with these specific cytokines we discussed so far and so forth.

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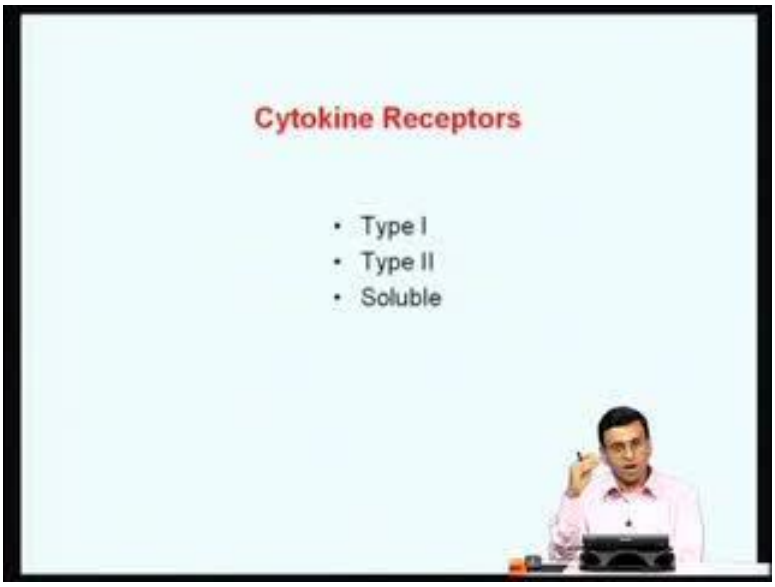

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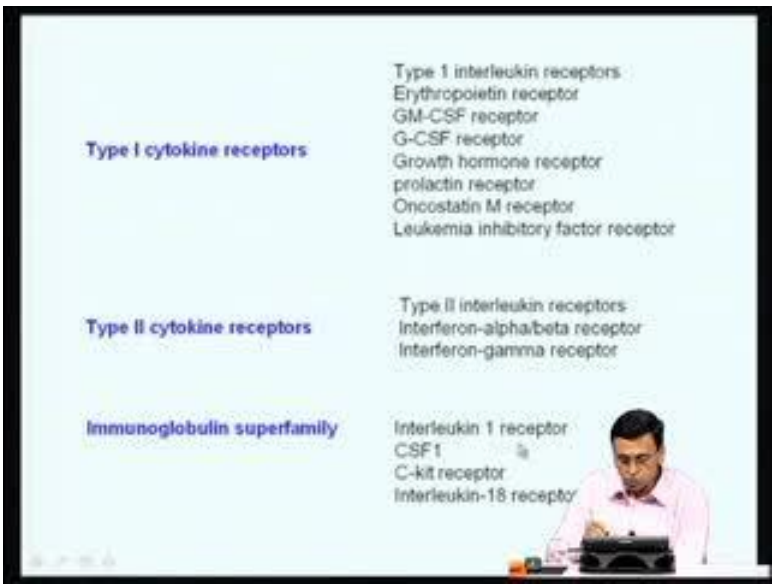

Now, there are three distinct types of cytokine receptors classified as type 1 receptors, type 2 receptors and soluble receptors. Now, examples for the type 1 cytokine receptors are type 1

interleukin receptors, erythropoietin receptors, granulocyte macrophage call estimating factor, GM CSF receptor, granulocyte call estimating factor GCSF receptor, growth hormone receptor, prolactin receptor, oncostatin m receptor and leukemia inhibitory factor receptor. So, all are very important receptors. They bind to very important ligands which has very important roles. So, all these receptors belong to the type 1 cytokine receptors.

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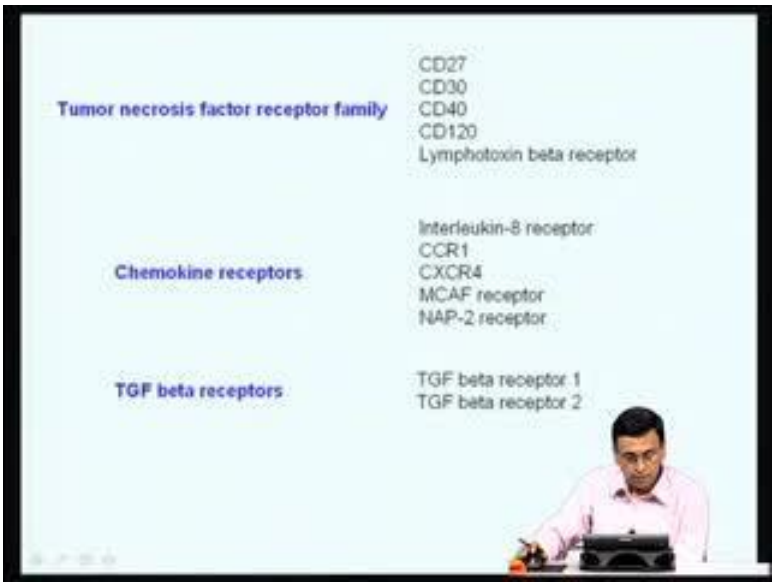

The type 2 cytokine receptors, some of the examples are the type 2 interleukin receptors, interferon alpha as well as interferon beta receptor as well as interferon gamma receptor. In addition to this, you have other types those belong to the immuno-globulin super family which include the interleukin 1 receptor, call estimating factor 1, CSF1, C kit receptor, the interleukin 18 receptor and you have what is called as a tumour necrosis receptor family which involve CD27, CD30, CD40, CD120 and lymphotoxin beta receptor.

We also have what are called as chemokine receptors which play a very important role in infection such as HIV. HIV in fact, requires many of these chemokine receptors, especially the CXCR4 acts as a co-receptor for HIV1 and without this receptor the HIV cannot transduce the signal further. Some of the chemokine receptors include interleukin 8 receptors, CCR1, CXCR4, MCAF receptor, NAP 2 receptors and finally, we have TGF beta receptors which include TGF beta receptor 1, TGF beta receptor 2.

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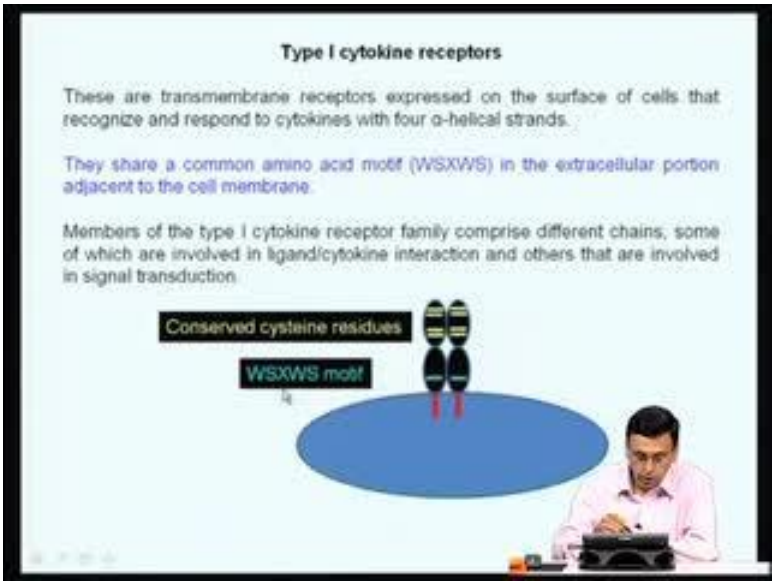

So, the cytokine receptors are of diverse cell types and some of these various cell types, we just discussed. Now, what are the distinction between this type 1 receptors and type 2 receptors? The type 1 cytokine receptors are basically transmembrane receptors expressed on the surface of cells that recognize and respond to cytokines with 4 alpha helical strands.

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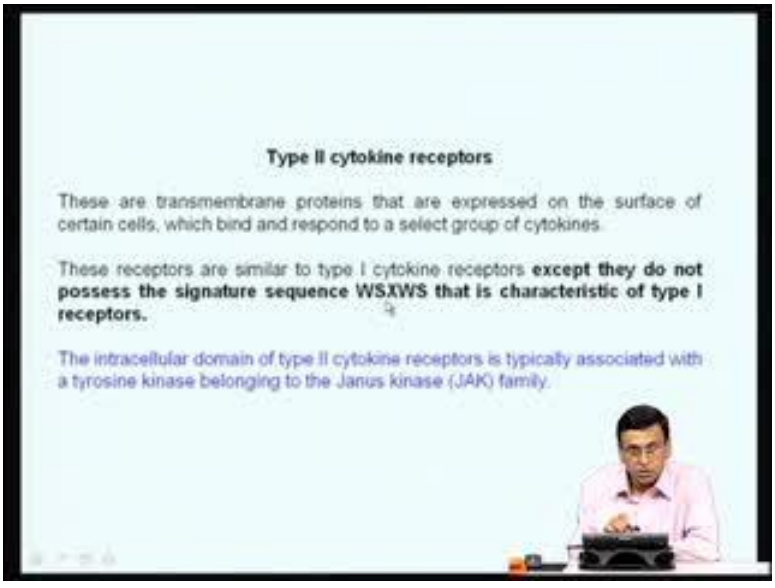

So, cytokine molecules which interact with the type 1 receptors contain 4 alpha helixes. They share a common amino acid motif called WSXWS in the extra-cellular portion of the adjacent to the cell membrane. So, I just listed here, this is the extra-cellular domain of the cytokine

receptor and this red one is the inter-cellular domain and you can see just the region adjacent to the cell where it binds the cell membrane, you have this WSXWS motive which is a signature motif for all the type 1 cytokine receptors.

The members of the type 1 cytokine receptor family comprise different chains. Some of which are involved in the ligand cytokine receptors and others are involved in signal transaction and in the far end of this extra-cellular domain, they also contain a number of conserved cysteine residues. So, these two presence of conserved cysteine residues as well as presence of the WSXWS motif is a very characteristic feature of this type 1 cytokine receptor.

The type 2 cytokine receptors are also transmembrane proteins. They are also expressed on the surface of cell types and they bind and respond to a select group of cytokines which we discussed earlier. These receptors are similar to the type 1 cytokine receptor except that they do not contain the signature motive which we saw in the type 1 receptors, namely the WSXWS motive which is very characteristic of the type 1 cytokine receptors is absent in the type 2 receptors.

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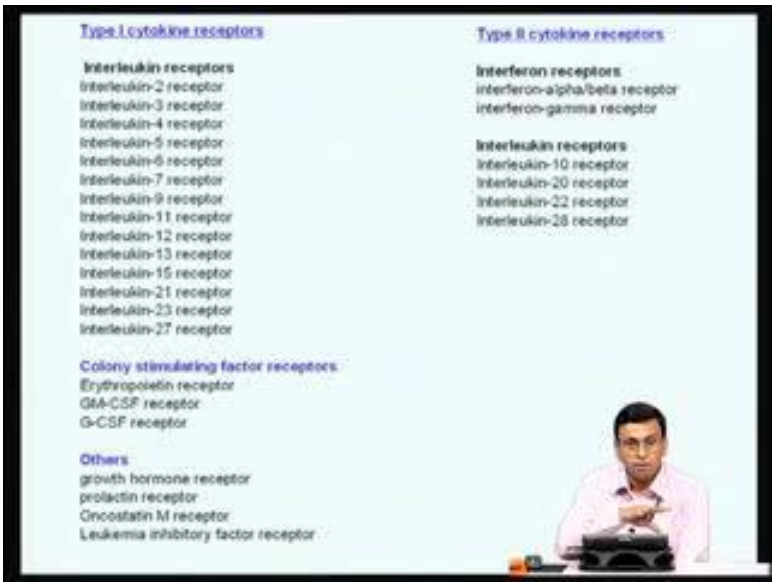

The other important thing about the type 2 receptors is that the intra-cellular domain of the type 2 cytokine receptors typically associates with the tyrosine kinase belonging to the Janus kinase family are known as popularly known as JAK family, the JAK kinase or Janus kinases. I just listed here the various cytokine receptors which belong to the type 1 cytokine

receptors. A number of interleukin receptors belong to the type 1 receptor and the type 2 receptors again interferon receptors both alpha, beta and gamma receptors as well as certain types of interleukin receptors belong to the type 2.

In addition, we have the colony stimulating factor receptors such as the erythropoietin receptor GM, CSF and GCSF and there are various other receptors which are also classified as cytokine receptors which include the growth hormone receptor, prolactin receptor, oncostatin M receptor and leukemia inhibitor factor receptor. So, these are the cytokine receptor family and mechanism that we are going to discuss today. All these receptors primarily act through the signal transaction mechanisms that we are going to discuss in the next few minutes.

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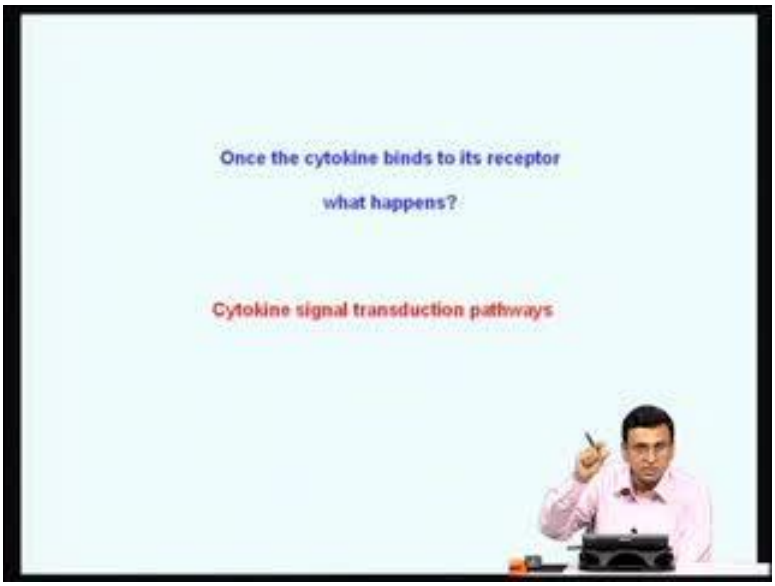

So, we have discussed so far what are the various cytokines? What is the importance of the cytokines? What are the functions of the cytokines and we also discussed for to the first step in the cytokine action namely, they go and bind to specific cells of phase receptors which are of different types like type 1, type 2 and so on and so forth. Now, ask the question. Once the cytokine binds to the receptor, what happens next? Once the cytokine binds to the cytokine receptor, then it initiates a specific signal transaction pathway.

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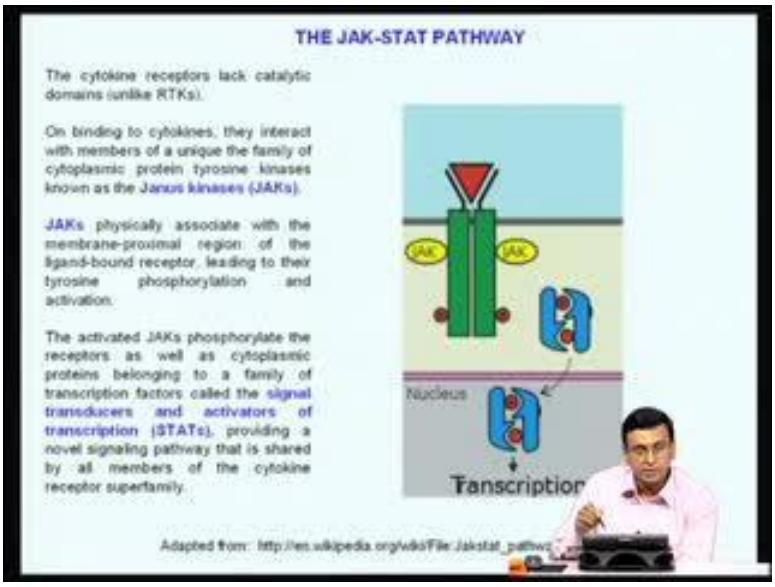

So, let us now understand what is this cytokine signal transaction pathway? What exactly happens once cytokine binds to a cytokine receptor? Now, the signal transaction pathways transduce by cytokines and cytokine receptors are popularly known as the JAK STAT pathway. Now, JAK stands for Janus kinases. These are the kinases which are the primary molecules which are attracted to the cell surface receptors once the receptor binds to a cytokine and the STAT refers to signal transducers and activators of transcription.

These are very unique kind of transcription factors that is very unique for the cytokine signalling pathway. So, let us now go in detail and understand what exactly this JAK STAT pathway is. Now, the important distinction that we have to remember before we go into the details is that the cytokine receptor that we have discussed so far of the type 1, type 2 and various other families of cytokine receptors, they are very different from the receptor tyro sonic kinases which we discussed in the previous class which are the growth factor receptors. In that, the growth factor receptors possess intrinsic tyrosine kinase activity whereas the cytokine receptors do not contain any tyrosine kinase activity. In fact, they do not have any protein kinase activity.

So, this is one important difference between the cytokine receptors and the receptor tyrosine. Kinases are growth factor receptors. Growth factor receptors are intrinsic kinases whereas cytokine receptors do not have any kinase activity. Now, then how do they transduce the signal? What happens once the bind cytokines bind to the surface receptor, they interact with members of a very unique family of cytoplasmic protein kinases known as the Janus kinases or JAKs.

So, in the case of growth factor receptors when the growth factor binds to the growth factor receptor, the tyrosine activity of the receptor itself is activated. So, the two monomers cross phosphorylate each other on the various tyrosine residues and this results in the activation of signal transaction pathway. Whereas, in the case of cytokine signalling when the cytokine binds to the cytokine receptor, it actually induces the recruitment of specific tyrosine kinases to the receptor and these kinases called as JAKs or JNS kinases.

Now, once the JAKs physically associate with the membrane region is what I have shown here is the cytokine, here is the receptor and here are the JAKs. So, once JAKs physically associate with the membrane proximal region that is the region of the receptor very close to the cell membrane, this results in the tyrosine phosphorylation. The JAKs cross phosphorylate each other and also they phosphorylate the tyrosine residues of the cytokine receptor which is shown in the next slide.

So, once the cytokine binds to the cytokine receptor, it recruits the Janus kinases. Janus kinases now are activated and these Janus kinases now phosphorylate the cytoplasmic domain of the cytokine receptor. Then what happens, the activated JAKs phosphorylate the receptors as well as the cytoplasmic proteins belonging to a family of transcription factors called signal transducers and activators of transcription.

So, once the JAKs phosphorylate, the cytoplasmic domain of the cytokine receptor these phosphorylated receptor now interacts with specific molecules known as the signal transducers and activators of transcription are abbreviated JAK STATs. So, these STATs are very unique group of transcription factors which are very unique and characteristics of the cytokine signalling pathway.

So, the JAKs phosphorylate the cytoplasmic domain of the cytokine receptor and this phosphorylated receptor now acts as a blocking site for the STATs and once the STATs go and bind to the phosphorylated tyrosines of this receptor, these STATs are again phosphorylated by the JAKs. Then these phosphorylated STATs now form either homo-dimers or hetero-dimers and then they go into the nucleus bind to distinct DNA elements of the enhancer elements and then activate transcription of specific genes.

So, you can see these cytokine signalling signal transaction pathway is quite different from the GPR signalling pathway and the receptor tyrosine signalling pathway which we discussed earlier. So, here we have a introduction of a very new mechanism involved in what are called

as Janus kinases which are cytoplasmic tyrosine kinases and another important group of molecules known as signal transduce in the activators of transcription of stats.

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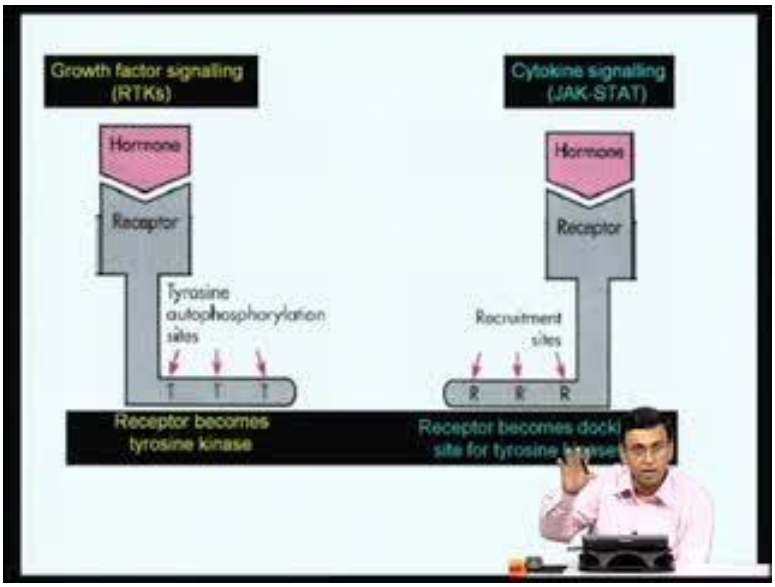

So, to emphasize this point what I mentioned, I just put a cartoon here just to tell you the two important distinctions between a growth factor signalling pathway and the cytokine signalling pathway. In the case of the growth factor signalling pathway, when the growth factor binds to the growth hormone receptor growth factor receptor, the receptor itself is a tyrosine kinase. It results in the auto-phosphorylation of the tyrosine. This is on the surface of the receptor tyrosine kinase and this in the activation of the receptor tyrosine kinase.

This phosphorylate tyrosines now act as a docking site for proteins like GRB2 and then through SOS, Ras is activated and then map kinase pathway is activated and transcription factors are phosphorylated into activation of genes. So, here the receptor itself is a tyrosine kinase whereas in the case of cytokine signalling pathway or the JAK STAT signalling pathway when the cytokine binds to the receptor, it acts the intra-cellular domain of the receptor, actually surface of the recruitment site or a docking site for tyrosine kinases and the receptor itself is not a tyrosine kinase.

Here the receptor is a tyrosine kinase; here the receptor only serves a docking site for a cytoplasmic tyrosine kinase. So, here when the hormone bind the receptor, the receptor becomes the tyrosine kinase whereas in the case of cytokine signalling when the cytokine binds to the cytokine receptor, the receptor becomes the docking site for a cytoplasmic

tyrosine kinase known as the Janus kinases or the JAKs. So, these are the two important differences between a receptor tyrosine kinase signalling pathway or the growth factor signalling pathway and the cytokine signalling pathway.

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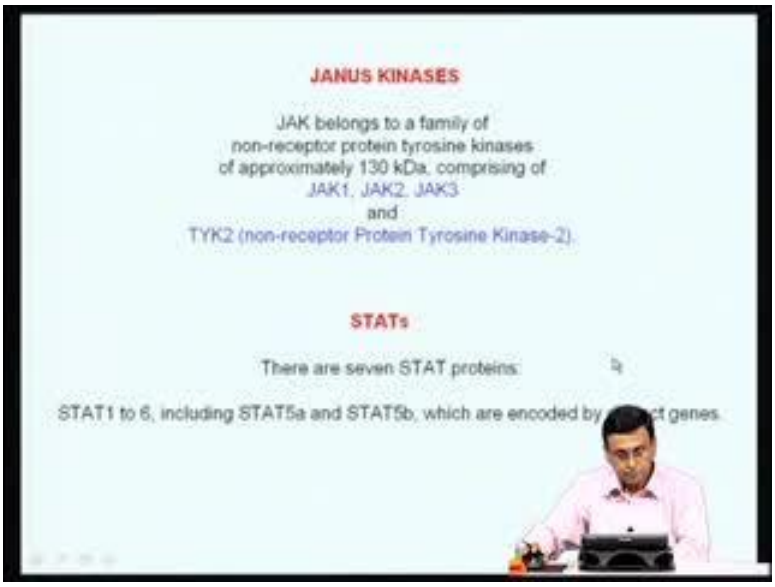

Now, so we have now studied about cytokines. We understood various cytokine receptors and then we told that once the cytokine binds the cytokine receptor, it results in the recruitment of a specific group of kinases known as the JAKs or Janus kinases. These Janus kinases then phosphorylate the receptor that results in the recruitment of the STATs. So, let us now spend some minutes to understand what are these Janus kinases? What are these STATs or the signal transducer activities of transcription?

Now, the Janus kinases or the JAKs belong to a family of non-receptor protein tyrosine kinases. This term is very important whereas the receptor tyrosine kinase or RT case are important transmembrane receptors for growth factor signalling whereas the Janus kinases are different from those so they are called as non-receptor protein tyrosine kinases. In fact, they are referred to other cytoplasmic tyrosine kinases which are recruited to the transmembrane receptor or else cytokine receptor.

So, the JAKs belong to a family of non-receptor tyrosine kinases of approximately 130 kilo dalton in size and they basically comprise of four different kinases known as JAK1, JAK2, and JAK3 as well as TYK2 known as the non-receptor protein tyrosine kinase 2.

So, JAK1, JAK2, JAK,3 and TYK2 these are the four kinases or four non-receptor cytoplasmic tyrosine kinases which interact with the cytokine receptors when these cytokine receptor interact with a cytokine molecule at the extracellular domain.

What was these JAKs do? The primary function of these JAKs is to phosphorylate the cytokine receptor and once the cytokine receptor is phosphorylated, the STATs are recruited to the phosphorylated cytokine receptor and then the JAKs phosphorylate the STATs also. Phosphorylations of these STATs are very important for the dimerisation and then their nuclear translocation and activation of gene expression.

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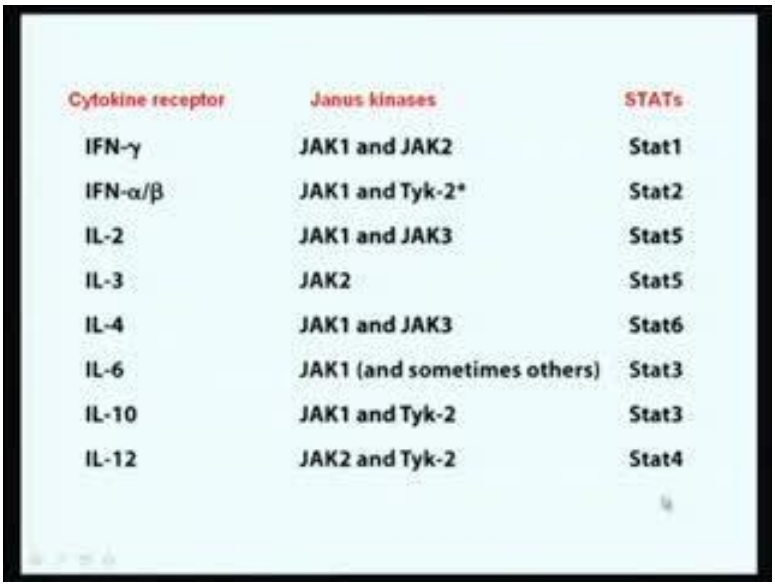

Now, the STATs, there are about seven different STAT molecules which have been discussed in mammal. These are known as the STAT 1, 2, 3, 4, 5, 6. STAT 1 to 6 this also includes STAT 5A and STAT 5B which are encoded by distinct genes. So, there are about seven different STAT molecules. Now, so in this particular slide, I have basically listed what kind of cytokine receptors interact with what kind of Janus kinases and ultimately, what kind of STAT molecules are activated.

So, if you now take interferon gamma, for example interferon gamma where interferon gamma interacts with interferon gamma receptor, it results in the activation of JAK1 and JAK2 which then phosphorylates interferon gamma receptor and recruits STAT1 to the receptor. Then STAT1 gets phosphorylated and then STAT 1 either forms homo-dimers or hetero-dimers STAT2 results and then goes in the nucleus and activates expression of

specific genes. Whereas, in the case of interferon alpha and beta instead of JAK1 and JAK2 JAK1 and Tyk 2 form are activated and these Janus kinases then come and activates STAT2. In the case of interleukin2, JAK1 and JAK3 are Janus kinases which interact with the a IL2 receptor and they activate STAT 5 whereas in the case of interleukin 3, JAK2 is activated and JAK2 then goes and phosphorylates STAT 5. IL 4 receptor primarily interacts with JAK1 and JAK3 and JAK1 and JAK3 then activates STAT6.

Whereas, IL 6, JAK1 sometimes other Janus kinase are also activated but primarily JAK1 is activated which then goes and phosphorylates STAT 3 and this in turn goes in nucleus and activates the expression of various genes. Whereas, IL 10 receptor serves as the docking site for JAK1 and Tyk2 which then activates STAT 3, whereas in the case of IL 12 JAK 2 and Tyk 2 are involved, they then activates STAT 4.

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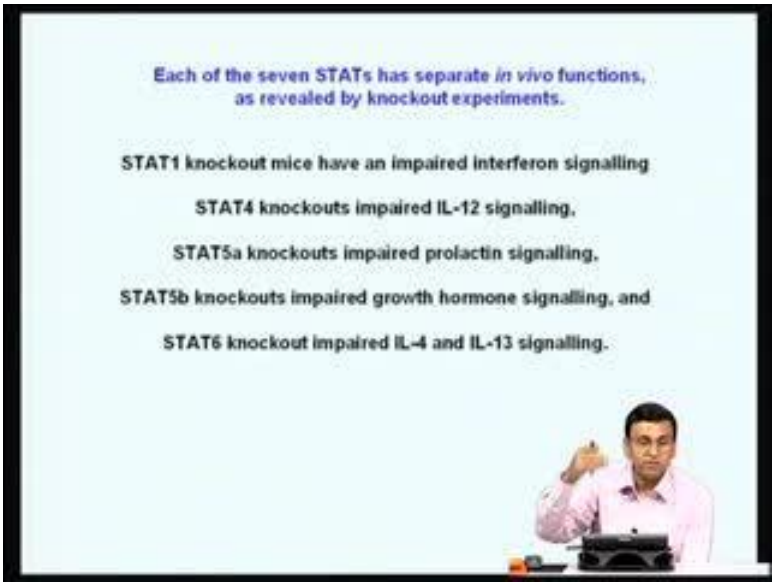

So, one can ask the question, why do you need so many STATs? They are all the same or they have different functions. The answer to this is in the next slide. Yes, you can see each one of the STATs I have discussed so far in the STAT1 to 6 and STAT5 and STAT b. Each one have a very important function, so then the organism does not have all these STATs for the same function but each one of them has a distinct function.

Now, how do we demonstrate that these type molecules have different functions? The best thing to understand a function of particular transcription factor is you create knockout mice. So, once you knockout this transcription factor, then you ask the question what happens to

the mice. So, if these STATs have redundant functions, then if you knockout one factor, the other factor will take over. It is function, so you should still be normal but if these STATs have non-redundant functions, then you should get specific phenotypes and that is what happens. You can see when you have STAT1, STAT1 is knocked out and these mice have interferon signalling. So, interferon signalling is drastically affected when you knock out STAT1 clearly indicating that STAT1 is very important for the interferon signalling.

When you knock out STAT4, IL 12 signalling is drastically affected. Nothing happens to interferon signalling clearly indicating that these two have different signalling functions or they are involved in different signalling transaction pathways. Similarly, if you knock out STAT5a, prolactin signalling is affected indicating that when prolactin binds to prolactin receptor, it results in the recruitment of STAT5 and all the physiological effects of prolactin is actually manifested is propagated by the STAT5 transcription factor.

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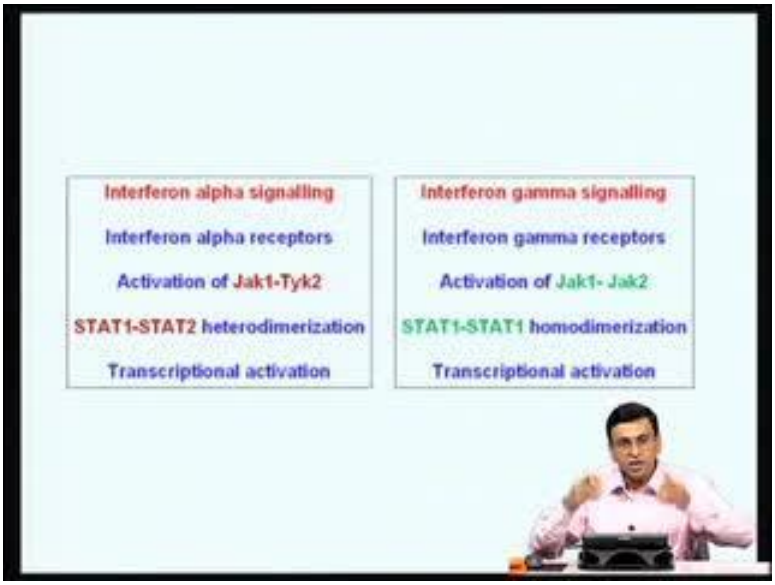

STAT5a knockouts have impaired growth hormone signalling. Similarly, STAT6 knockout have impaired IL 4 and IL 13 signalling. So, this basically tells you that each one of these STAT molecules go and activate different set of target genes and that is why you have different physiological responses in response to the initial signalling molecule through which they got activated. Just to give you an example here of the various things we have discussed so far.

If you take for example, interferon alpha signalling. Interferon alpha goes and binds to interferon alpha receptors and when interferon alpha binds interferon alpha receptor, it results in the activation of the JAK 1 and type 2 and these Janus kinases, then phosphorylate the interferon receptor and when the interferon alpha receptor is phosphorylated that becomes a docking site for STAT1 and STAT2. These again get phosphorylate by JAK1 and Tyk2 and this phosphorylated STAT1 and Tyk2 now form hetero-dimers. Then they go into the nucleus and activate distinct set of genes.

Whereas, in the case of interferon gamma signalling where interferon gamma binds to interferon gamma receptor that results in the recruitment of JAK1 and JAK2 whereas in the case of interferon alpha, it is JAK1 and Tyk2. Now, when JAK1 and JAK2 are recruited into the interferon gamma receptor that results in the phosphorylation of the interferon gamma receptor that results in the recruitment of STAT1 but not STAT2 and these STAT 1 now form homo-dimers. They in turn go and bind to distinct set of elements and activate the transcription of specific target genes.

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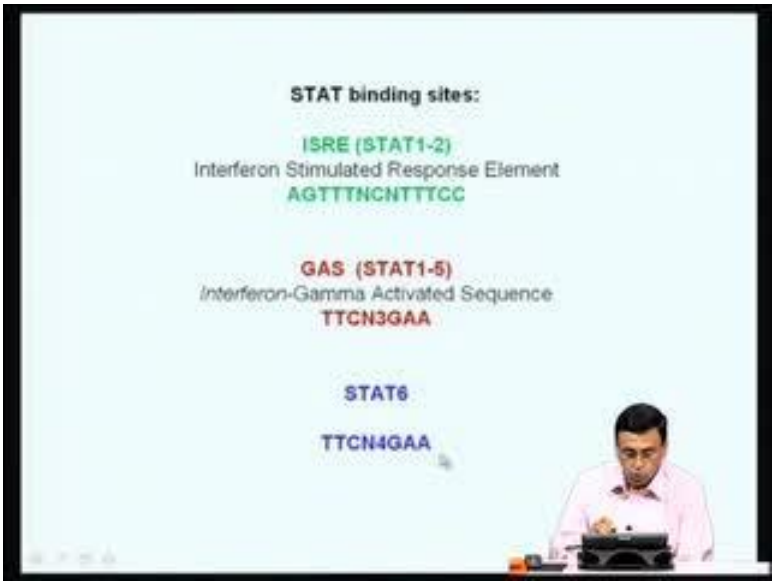

So, despite of the fact that these cytokine receptors acts through Janus kinases and various STAT molecules, there are differences as what kind of Janus kinase are recruited to the activated receptor as well as what kind of STATs are activated by these Janus kinases? This difference is what manifests each cytokine signalling as a different pathway. The STAT binding sites in the promoters of various target genes are also quite distinct. For example, the

STAT12 binds to what is called as the interferon stimulated response element are known as ISREs and this is the nucleotide sequence to which the STAT 1 hetero-dimer goes and binds.

So, the target sequence for the STAT 12 is known as ISRE and this is the particular sequence to which this binds. Whereas the binding sequence, the target sequence, recognition sequence for the STAT 15 transcription factor is known as gas or interferon gamma activated sequence and this is shown as the concession sequence of gas is shown here TTCN3GAA and in the case of STAT 6, it goes and binds a specific sequence TTCN4GAA.

So, you can see here it is TTCN3GAA whereas for STAT6 it is TTCN4GAA. So, each one of these STATs are activated by different Janus kinases and which in turn are interacted with different kinds of cytokine receptors and these STATs go and bind to distinct response elements in the target genes. Therefore, distinct different target genes are activated in response to each one of the STAT molecules. That is why when you knock out these STAT molecules, you get different kinds of physiological responses because they bind to they go and activate different kinds of target genes.

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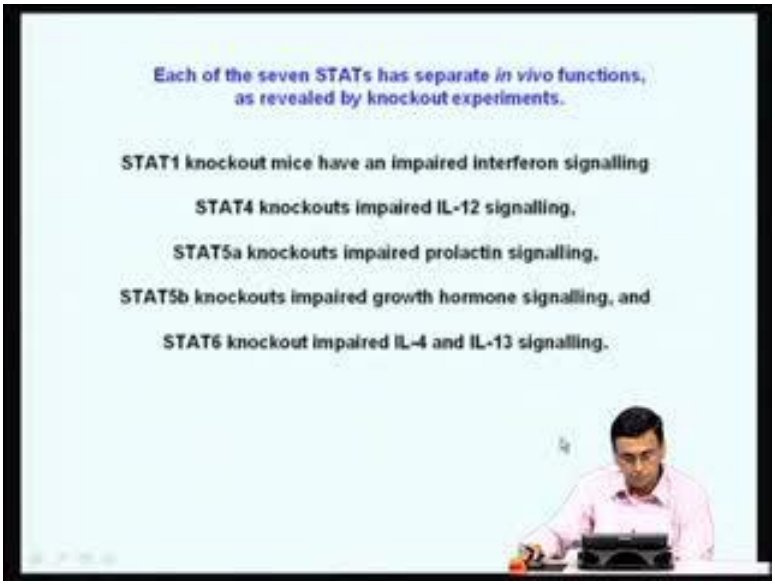

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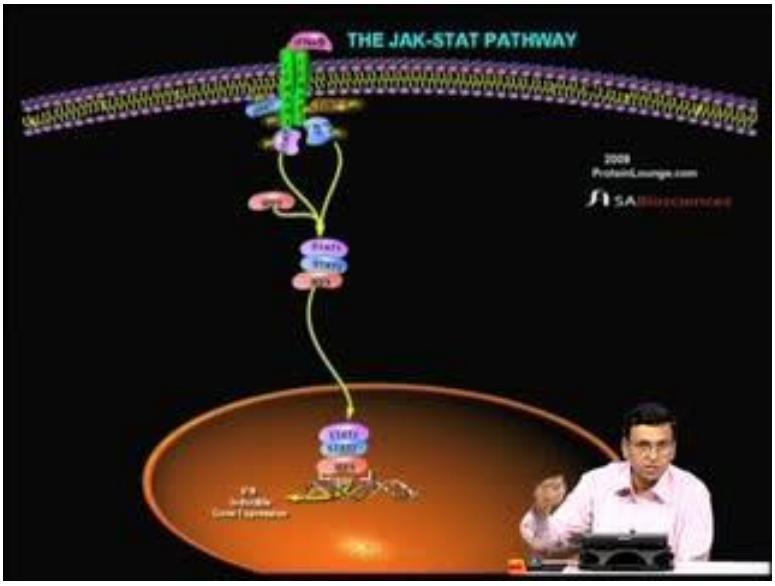

So, what I have now done is I have just drawn some cartoons. These are all taken from the SA biosciences pathway central. Very nice cartoon just for you to get an understanding how each one of these cytokines activate expression of various target genes. For example, if we take the interferon alpha or beta, they go and bind to the interferon alpha receptor which is a homo-dimer and when interferon gamma binds to the interferon alpha receptor, it results in the activation of the JAK1 and Tyk2.

The JAK1 and Tyk2 then phosphorylate the interferon alpha receptor and once the interferon alpha receptor is phosphorylated that now recruits STAT molecules, especially STAT1 and STAT2. Once STAT1 and STAT2 are recruited, they are again phosphorylated by the JAK1 and Tyk2 and this phosphorylation of the STAT results in the formation of hetero-dimer. Many times another transcription factor IRF9 also joins the party and these together go inside the nucleus and bind to the interferon stimulated response elements or ISRAs and activate the transcription of specific target genes. This is how physiological responses for interferon alpha and beta is brought about.

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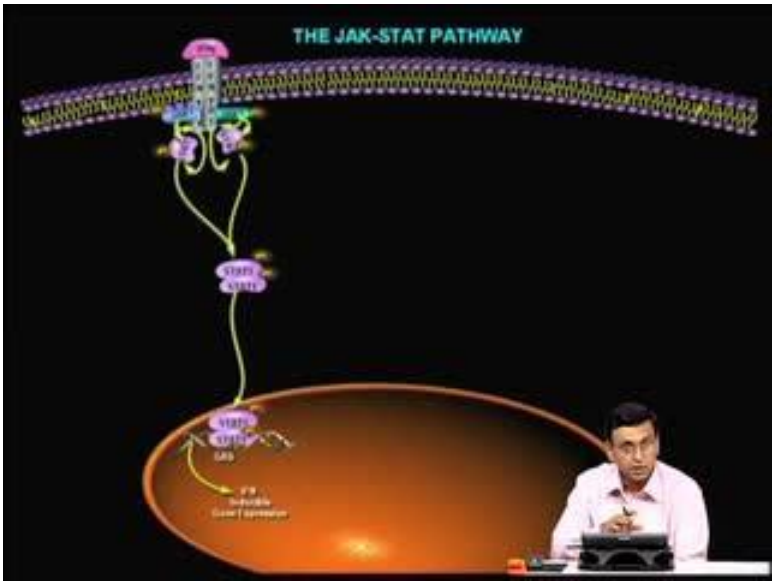

So, binding of interferon alpha and beta to the interferon alpha receptor triggers a pathway involving STAT1 and STAT2 and all the target genes soon contain these ISRAs are now bound by these. They activate the expression of the genes leading to specific interferon alpha specific responses whereas if you go to the interferon gamma, in the case of interferon gamma when the interferon gamma binds interferon gamma receptor, it instead of in the interferon alpha it was JAK1 and Tyk2 but here, it is JAK1 and JAK2.

These two of Janus kinase are now recruited to the receptors and they phosphorylate the receptor and this phosphorylated receptor now serve as a docking site for STAT1 homo-dimers and when these STAT 1s are phosphorylated by the JAKs, the phosphorylated STAT1 now forms a homo-dimer. They go inside the nucleus and bind to interferon gamma or the gas sequence of target genes and then activate different set of target genes. So, all the genes which contain the interferon gamma activating sequence of the gas motives now get activated by the STAT 1 transcription factor. So, in the case of interferon alpha, it was JAK1 and Tyk2. Here it is JAK1 and JAK2 and there it is STAT1 STAT2 hetero-dimer, here it is STAT1 homo-dimers and the response elements are also quite distant.

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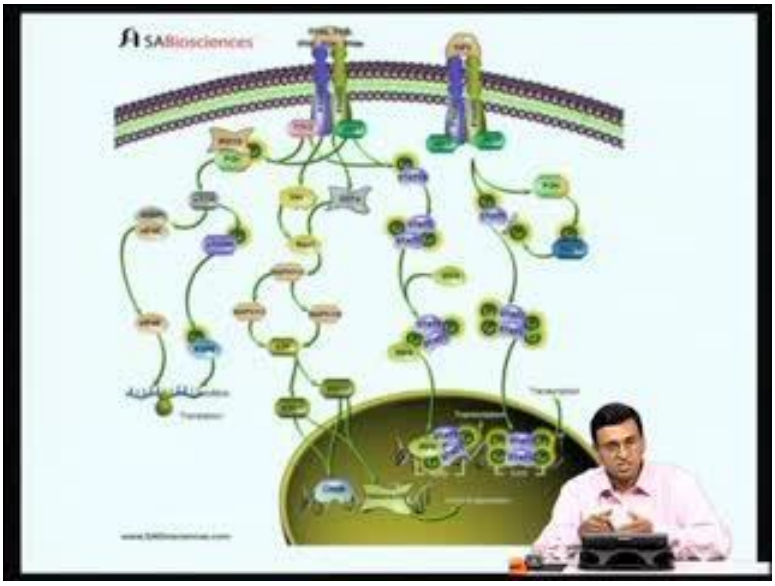

So, these slides summarize both the pathways. The interferon gamma primarily activates the JAK2 and through this the STAT1 homo-dimers are activated which go and binds to the gas sequences of target genes. Whereas, in the case of interferon alpha the STAT1 STAT2 hetero-dimer is activated which now goes and binds the ISRE sequences and they activate the transcription of further genes.

Now, the signal transcription pathways are always complicated and you can see here in addition to the things. There is also a cross talk involved in PI3 kinase which is also activated which then activates protein kinase C that in turn can phosphorylates STAT and then it can lead to activation of the STAT. Similarly, the activation of interferon alpha signalling pathway involving Janus kinases can also go and then activate PI3 kinase which again results in the a different kind of signalling molecule leading to activation of different kinds of metabolic enzymes.

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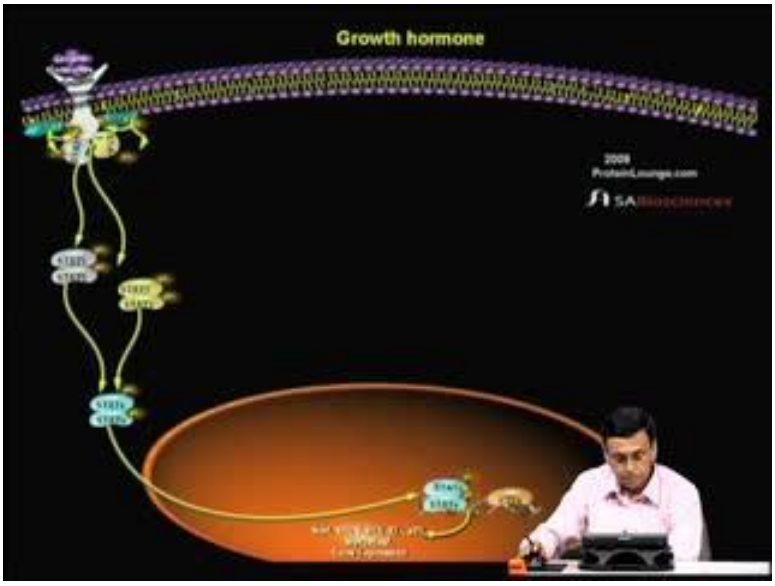

Since, our focus is primarily on the gene expression, we will not worry about the events which take place outside the nucleus when these pathways are activated. So, we are going to confine our self to what are the events which happen inside the nucleus when these signal transaction pathways are activated. Here again, another example of growth hormone, we need growth hormone for our regular growth.

Now, how does growth hormone promote growth? Growth hormone binds to a growth hormone receptor and when growth hormone binds to growth hormone receptor, again the STAT3 and STAT5 are recruited to the phosphorylated receptor and JAK2 is the Janus kinases which involved here these STATs again go into the nucleus and bind to growth hormone. The growth hormone responds to genes by binding to specific targeting sequences and then activating transcription of the genes. This results in the proliferation of growth of the organism.

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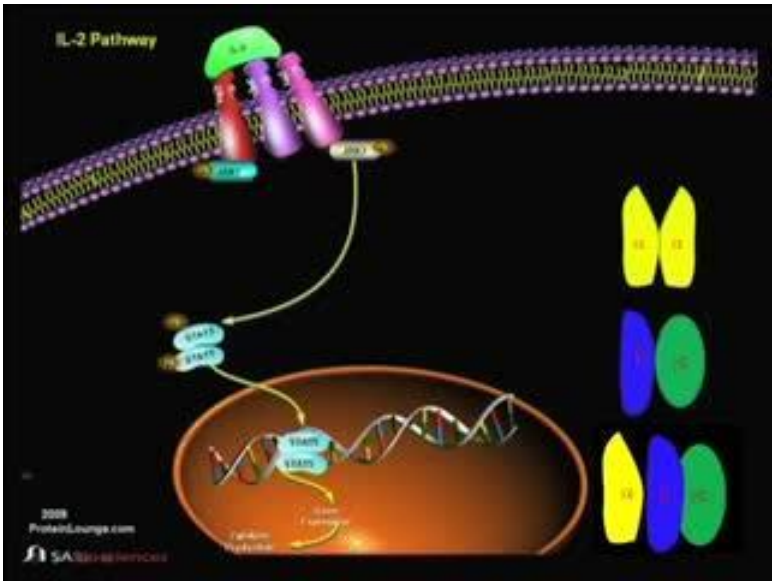

So, the physiological response of growth hormone is mediated by this particular cytokine signalling pathway involving STAT3 and STAT5. In the case of interleukin 2 for example, the interleukin 2 is slightly different from the interferon alpha and beta signal transaction. We have discussed far here interleukin receptor often is a trimmer consists of alpha, beta and gamma subunits and then the interleukin 2 bind to this interleukin 2 receptors. Again, it results in the recruitment JAK1 and JAK3.

You can see here we are taking a different kind of a JAK combination here. JAK1 and JAK3 get activated. They get phosphorylated when the IL2 docks the IL2 receptor and this JAK now phosphorylate the receptor and then also phosphorylates STAT5. This STAT5 now forms homo-dimers and these STAT5 homo-dimers now go inside the nucleus and they bind to specific sequences and activate the expression of genes and especially, it leads to the production of various cytokines.

So, you can see different JAK kinases are involved in activation by different cytokines and different STAT molecules are involved and different kinds of target genes are activated. This is the signal transaction pathway by which IL2 interferon alpha, beta, growth hormone interferon, gamma activates specific target genes involving specific Janus kinases and specific STATs. Now, very interestingly the interferon the IL2 receptor can exist either as a homo-dimer involving 2 alpha sub units or as a hetero-dimer involving beta and gamma sub units gamma and c sub units and you can also have all the three sub units together.

It turns out, they can exist either in the homo and hetero-dimers form and then when you have only the interferon, when you have only the alpha sub unit homo-dimers, the affinity of the IL2 to IL2 receptor is very low. It is something like 10 to the minus 8 molar and this kind of homo-dimers are expressed only by activated T cells and because of the very low affinity they have no function at all.

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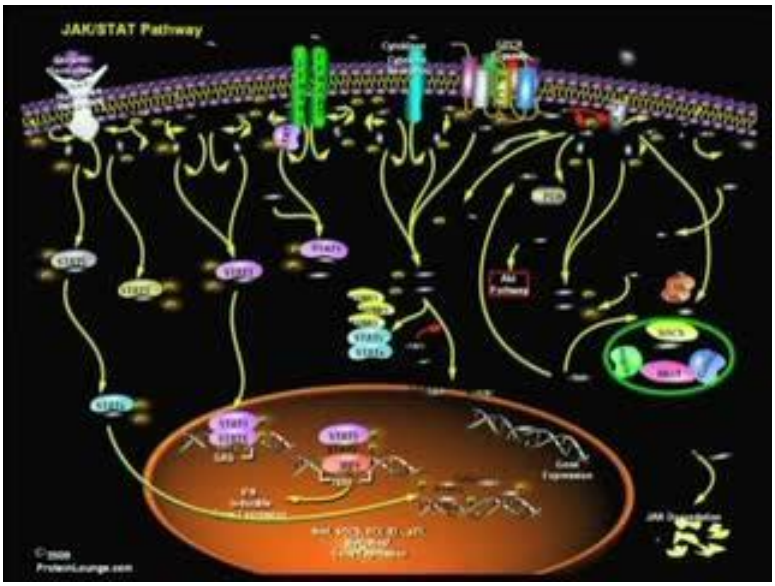

Whereas, when they exist as hetero-dimers with beta and gamma and c, the affinity slightly goes up there. It is now tend to the minus 9 molar and such kind of IL2 receptor hetero-dimers are found in resting T cells. The affinity is termed as intermediate whereas the high affinity interleukin receptors involving the trimmer, the hetero trimmers have very high affinity and these are the ones which actually play very important role and they are present in the activated T cells.

So, this just summarizes the various JAK STAT pathways. Again, I will discuss to the growth hormone receptor pathway. I have discussed the interferon alpha pathway and the interferon gamma pathway and basically, this slide I do not expect it understand all these things. The slide is primary to tell you that in addition to each receptors acting in a specific STAT pathway, you can also see this lot of cross talk.

Sometimes, the activation of the GPS signalling pathway or a receptor tyrosine kinase pathway can also lead to the phosphorylation of tax and all of them can also together activate target genes involving a cumulative effect leading to ultimate activation or repression of

various target genes. So, signal transaction pathways are never isolated. So, when we talk about interferon gamma signal through STATs, it does not mean that other signalling pathways are not activated. Ultimately, a target gene is activated by not only through one particular signalling pathway but also due to cross talk between numbers of other signalling signal transaction pathways. So, the ultimate expression of a gene depends on signal received from multiple signal transaction pathways and that is of the sum total of all these events, ultimately manifest as a form of an activation or repression of a specific target gene.

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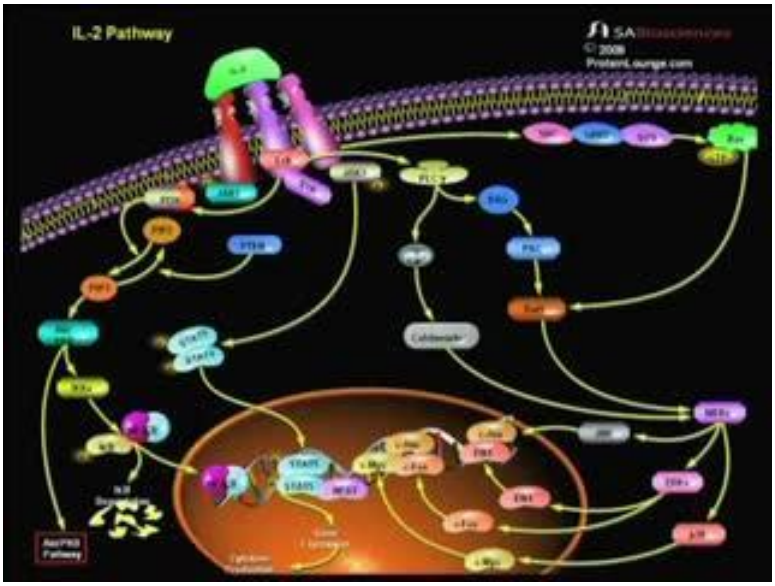

This is again the IL2 pathway again tells you in addition to the activation of the STAT5, you can also there is an activation of NF Kappa b and you can also see there is a phosphorylate C gamma pathway that is involved resulting in the activation of map kinase pathways in the activation of the AP1 or the CG units c phos and you can see ultimately the target genes are activated not only by the STATs but also by NF Kappa b c (( )) , c phos, c (( )) as well as various other transcription factors.

So, many times the activation of a target gene depends not only on one particular signal transaction pathway but also because of cross talk between numbers of other signal transaction pathway. Simultaneously, a number of transcription factors are activated by receiving signal through different signal transaction pathways leading to very high level of activation of a particular target gene.

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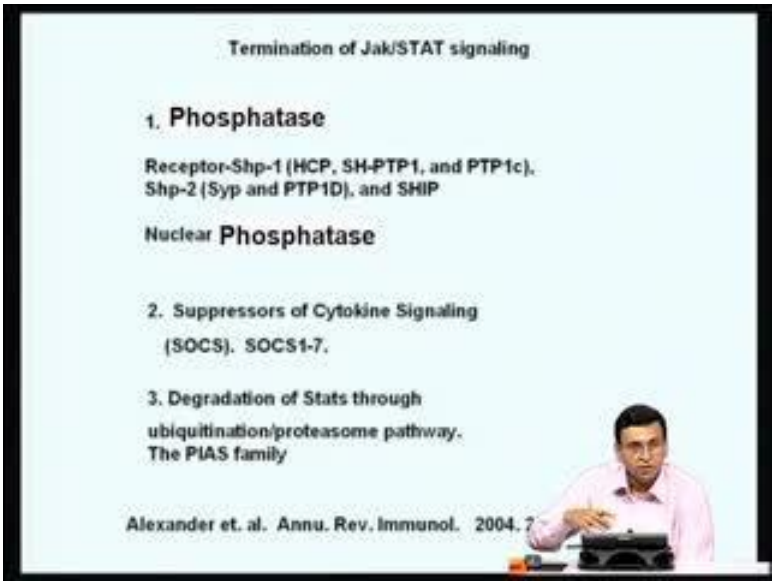

How do the once the JAK STAT pathway is activated, how is it terminated? Again I will not go into details. It is again a very interesting and a lot of network that operates here. It involves two important proteins known as the SHP1 and also the SOCS or suppressors cytokine signalling. When these are activated, it results in the degradation of the STATs involving ubiquitination and proteasome pathway involving a group of proteins belong to the PIAS family but I will not go into the details of how once the JAK STATs are activated and how this pathway is terminated.

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Those of you who are interested can read this external excellent review by Alexander et. al. when the annual review of immunology in the 2000 year 4 volumes 22 page 503 gives you a very good example. I have reviewed of how these JAK STAT pathways is inhibited once it is activated by through these molecules.

Now, so what we have discussed so far is how cytokines which are very important molecules when they dock to cytokine receptors, recruit Janus kinases and when these Janus kinases are recruited, it results in the activation of the STATs. Different kinds of STATs either form homo-dimers or hetero-dimers and then they go inside the nucleus and bind to specific response elements and activate the transcription of various target genes.

Now, the title of this course is eukaryotic gene expression basics and benefits. So, whenever I discuss some of these important topics, I always try to put some information on how understanding the signal transaction pathways or understanding how specific genes are activated by the signal transaction pathways has helped the mankind or has helped to bring about new drugs or new therapeutic agents.

For example, I am going to show some couple of slides here to tell you how very important drug molecules have been brought about based on our understanding of the cytokine signalling pathway. A very popular dock known as Enbrel is nothing but it is a monocline antibody against the TNF alpha receptor. So, the Enbrel basically is a monocline antibody. It prevents TNF interacting with TNF receptor and therefore the TNF signalling pathway is not activated. This Enbrel is a very important drug used in the treatment of rheumatoid arthritis as well as in auto-immune disease called Crohn’s disease.

So, you can see how molecules which interfere with the signal transaction or the cytokine signalling pathways can be very important drug molecules. Another example, we have molecule intron a, which is nothing but interferon a2b which is very widely used in the treatment of hepatitis c as well as certain melanomas. Epogen is nothing but recombinant erythropoietin. The erythropoietin is very important stimulators of red blood cell production. So, if you have anaemia or if you are undergoing kidney failure patients who cannot produce erythropoietin, erythropoietin is given to stimulate the red blood cell production.

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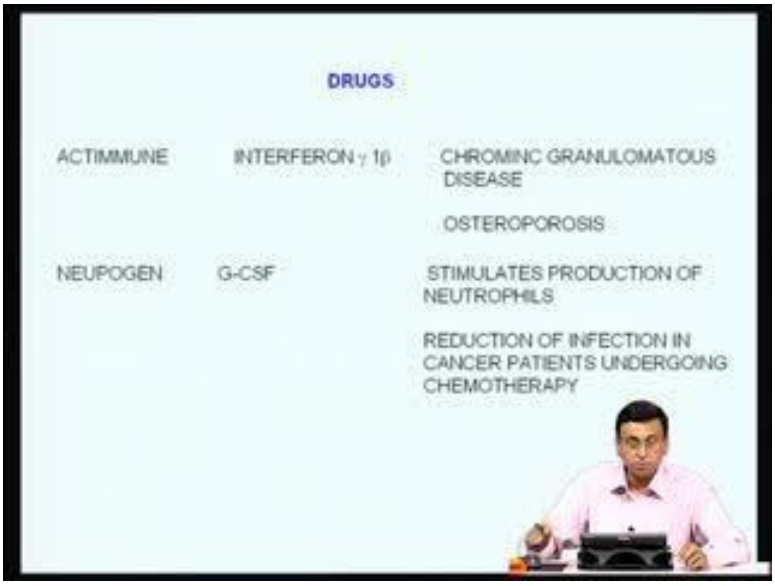

So, it is a very important activator of erythropoiesis. All of them act through the JAK STAT signalling pathway. Similarly, act immune is again nothing but interferon gamma 1 beta again used in the treatment of chronic granulomatous disease as well as osteoporosis. Yes, one of the drug neupogen which is nothing but re-combinant garanulocyte colony estimating factor. It stimulates the production of neutrophils reduction in the infection in cancer patients who undergo chemotherapy.

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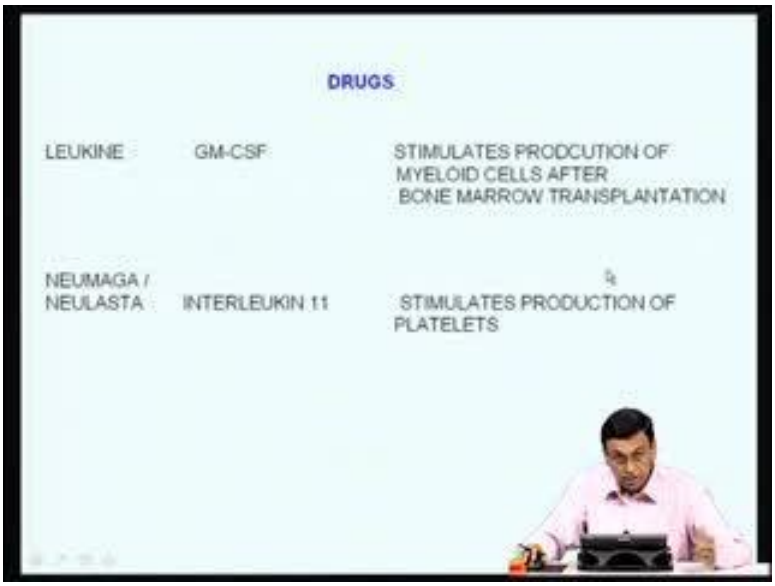

Cancer patients undergo chemotherapy because the immune cells are also getting destroyed. They are very susceptible to a various infections. So, such patients are given GCSF, actually to promote the production of the immune cells or the T cells and B cells, so that they can become less susceptibility to various infections. So, GCSF is very important molecule here. Similarly, GM CSF is marketed in the trade name leukine. It actually stimulates the production of myeloid cells after bone marrow transplantation.

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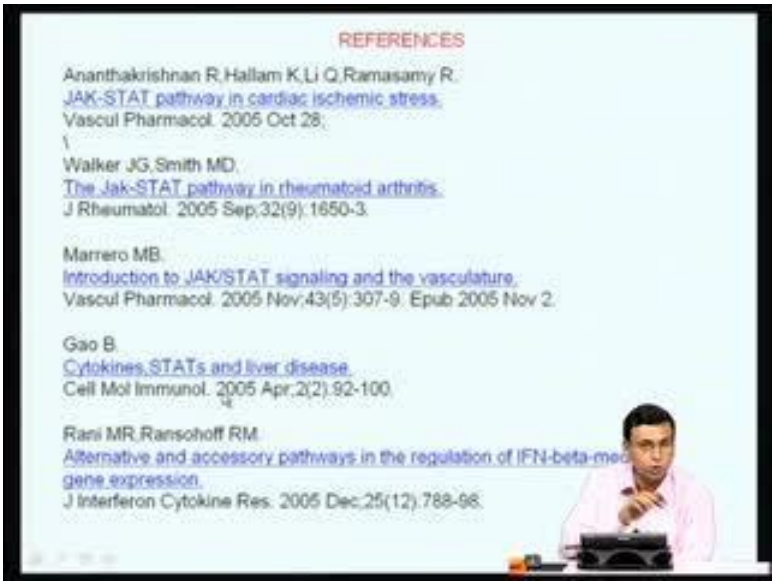

Neumaba or neulastia is nothing but re-combinant interleukin 11. It actually is used in the stimulation of production of platelets which helps in blood clotting. So, what we have discussed so far is cytokine signalling is very important and the cytokine family is a huge group of molecules of diverse molecules ranging from interferons, interleukins, erythropoietin, GM CSF, growth factor, growth hormone and so on and so forth. Many of them act through non-receptor tyrosine kinases and when these molecules bind the cells surface receptor and especially, they recruit specific Janus kinases which interact with molecules called STATs.

These STATs which again are diverse type of molecules having non-overlapping functions, they in turn go and bind to specific target elements of target genes activate or repress transcription of various genes. I have now listed a number of references here again taken from a very important website promoted by the SA biosciences called pathway central. If you

read some of these references, it gives you a much more appreciation of how important these cytokine signalling pathway in controlling a number of diseases.

For example, the JAK STAT pathway in rheumatoid arthritis gives an excellent review of how understanding the JAK STAT pathway and they are how these pathways are activated in rheumatoid arthritis. People are trying to develop various drugs for treatments of arthritis, cytokine STATs and liver disease again correlating how basic research can be used for development of various drug molecules. Again, inhibition of interferon stimulated JAK STAT signalling by tick borne flu virus and identification of NSS as an interferon antagonist.

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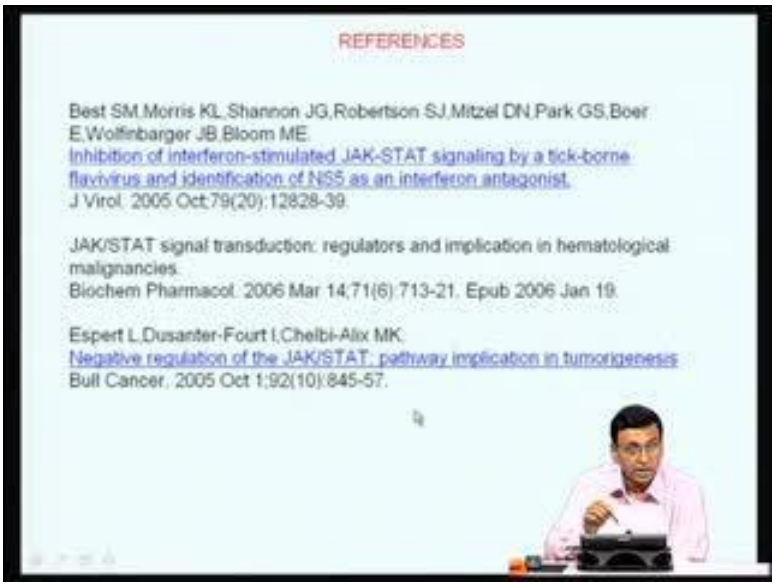

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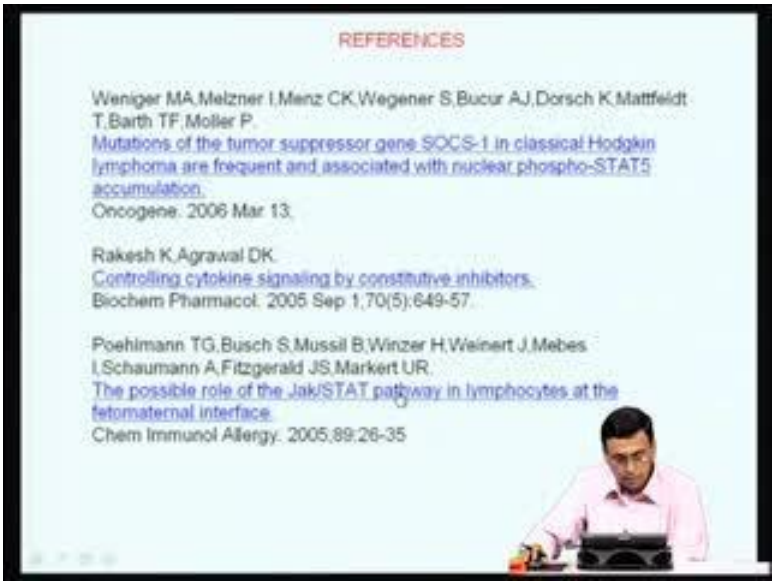

How JAK STAT pathway is activated in specific viral infections and how you can actually use? How viruses actually interfere with these interferon signalling pathway and then they way the immune system, so that they can survive inside the cells. The role of JAK STAT signalling in cancer as well as the last two actually how JAK STAT pathway plays a very important role in role in number of cancer. A few more references basically to give an idea how important are these, especially this article discusses about how the JAK STAT pathway is actually inhibited by the SOCS one pathway.

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I would like to acknowledge that many of those colourful pathway slides I have shown you so far. I have all been taken from the SA biosciences pathway central, a beautiful website for understanding a number of signal transaction pathways and you can just click on or cut paste this web link and you can go and then you can just have to logon, put your user name and give a password. It is a free website, people or any academic researcher can actually use this to understand various signalling pathways and very colourful slides and very colourfulness illustrations are given to understand how various signalling molecules activate this distinct signal transaction pathways leading to activation or repression of specific target genes.

So, I think we will stop here. So, what we have discussed so far in this series of signal transaction lectures about 5 lectures from lecture 15 to lecture 20. We basically covered one aspect of signal transaction pathways. We primarily talked about molecules which interact with G protein couple receptors which activate through receptor tyrosine kinases and those which now activate through the JAK STAT pathway. There are many other membrane receptor molecules. There are many other pathways by which various membrane receptors are act through but it is not possible to cover all these things.

So, I have just given you a very brief idea of how molecules which cannot enter the cell can bind to specific membrane receptors and they these three examples tell you how binding of these molecules membrane receptors can transduce signals either through map kinase signalling or through a specific protein kinases like protein kinase c or protein kinase a in the last case. We have discussed today the JAK STAT pathway, ultimately results in the activation or repression of specific target genes manifesting in the form of distinct physiological responses.

With this we have completed the signalling and regulation of gene expression by molecules which bind to cell surface receptors but I strongly encourage you to go and read up a few more interesting examples like transform a growth factor beta, erythropoietin, hypoxia inducing factor. These are all wonderful examples to understand how this membrane signalling works and how each one of these receptors bring out distinct physiological responses by activating different kind of protein kinases and so on and so forth.

They all have very important biomedical relevance. What we will do in the next class? We will now move inside the cell. We will now talk about molecules which actually diffuse through the cell membrane but bind to a specific intra-cellular receptors and then when these

signalling molecule or the hormone receptor complex, then goes inside the nucleus in the activated cell transcription. So, far we remain in the outside the surface talk about molecules which interact with membrane receptors on the cell surface. From next class onwards, we are going to move inside the cell and talk about molecules which go inside the cell, interact with specific intra-cellular receptors and then activate or repress the transcription of specific target genes. I will stop here.