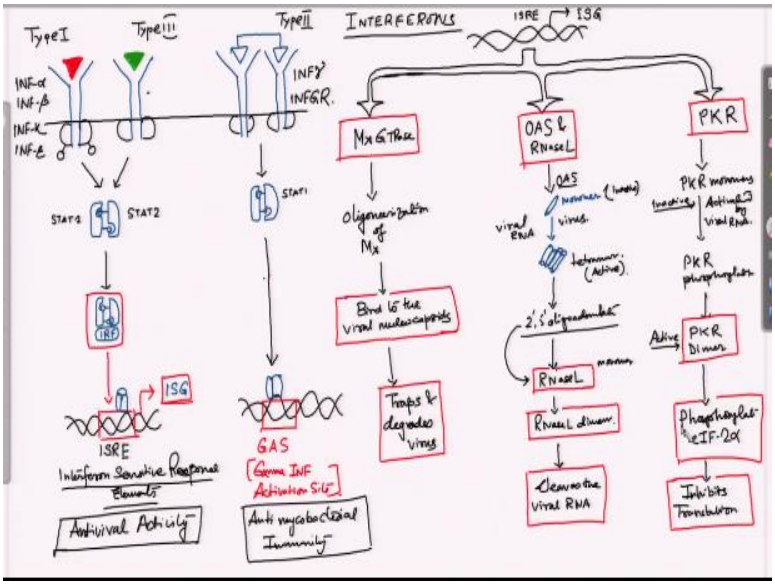
Immunology
Prof. Agneyo Ganguly
Department of Biotechnology
Indian Institute of Technology-Kharagpur

Lecture - 48
Interferons

So welcome to the immunology lectures and today we will discuss about the interferons.

(Refer Slide Time: 00:23)



As I told in my last class, where we stopped in the last class we have talked about the cytokines for quite a long time now in at least in three lectures. So what these cytokines are? Then we discussed about the different receptors, the cytokine receptors and the classification of the cytokines, how they are classified mainly depending on their functions and depending on the receptors they bind to.

And then we have discussed about the different actions of the cytokines or the different types of cytokines that are that act in the innate as well as in the adaptive system. A specific class of cytokine is the interferons. Interferons falls under the class of cytokines. And they also have the same mode of action, they bind to similar cytokine receptors. And they have almost similar mode of action like activation by this JAK and STAT pathway that we had discussed in our in cytokine receptor class.

So this also leads to STAT dimerization and then upregulation or expression of certain genes downstream. So why interferons? Interferons are broadly classified into three different classes. There is a type I, the type II and the type III. That type II interferons falls under a very different class. Why? Because the type I and the type II, the type I and the type II are the two major class of interferons that are involved in antiviral activities.

So interferons remember, are the primary molecules that are responsible to fight viral infection. So whenever there is a viral infection in our body, the interferons are the first molecules to react. And there are certain interferon sensitive regulatory or response elements, which are present on our gene sequences and that are being transcribed in response to the interferon binding.

So starting from where we had been discussing in the cytokine receptor class if you remember, in the cytokine receptor class, we discussed about the receptors, the cytokine receptors and the cytokine receptors as we told that usually when there is dimerization of this receptor, so the receptors normally they remain as monomers and when there is binding of a cytokine, a specific cytokine there is dimerization or tetramerization or oligomerization of this receptors.

And that leads to the, that activates this kinases, which are the JAK kinases, the Janus Kinases. And this JAK kinases they start phosphorylating the receptors. They are tyrosine kinases associated with this receptors and they start phosphorylating the receptors as specific sites. And due to this phosphorylation then, there is docking of the STAT molecules.

The STAT molecules, which are normally present as monomers, they then come and bind or docks onto the receptor. Now when these STAT molecules they dock onto the receptor, they are then cross phosphorylated by the JAKs or the Janus Kinases. And this STAT gets phosphorylated. Now once this STAT is phosphorylated, the phosphorylated STAT can now dimerize.

So then it forms a dimer and this phosphorylated STAT, once it dimerizes the dimeric STAT can now go into the nucleus and can upregulate or induce expression of several

genes. Now the same thing or the same mechanism is also applicable in case of the interferons. If you look into this picture here, the interferons I have classified type I and type II together. So they kind of have similar receptor structures.

And they also have the similar JAKs or the JAK Kinases and the similar STATs that are responsible for mediating the downstream signaling. The type I and the type III and both these type I and type III interferons, they are involved in antiviral responses. The wide class of interferons or the widely classified interferons are the type I.

So the type I interferons are primarily the interferon alpha, beta, kappa and epsilon of which interferon alpha is the dominating interferon. It is everywhere and is the primary interferon. And its binding to the receptor can elicit a downstream signaling. The class II or the type II interferons, they belong to a very different class. And in that case, it leads to a receptor tetramerization.

So it binds to the receptor like this, which brings two dimers together. So there is two dimers coming together. And it is a different class of JAK that are associated with this type II interferons. And these receptors are usually known as the interferon INFGR, that is the interferon gamma receptors, and they are very specific for the type II or the interferon gamma, the type II interferons or the interferon gamma.

So they are primarily or they are very specific for the type II interferons or the interferon gamma type. Now there is also the downstream signaling is also pretty much similar. That is, it is a STAT mediated signaling. So that also leads to dimerization of STAT I, and it forms a homodimer of STAT I. In case of type I, and type III interferons, it is a heterodimer of STAT I and STAT II.

So it is the same STAT, but it is STAT I and STAT II, which gets phosphorylated. And then they come together and they forms a dimer. Now this dimeric STAT, this dimeric STAT then associates with an interferon regulatory factor or the IRF. IRF this together this complex along with the STAT the dimer and the IRF, this can then go and bind to specific sequences in the DNA.

And these sequences are known as ISRE that is interferon sensitive response elements, interferon sensitive response elements. Now this interferon sensitive response element, this is the DNA element, element on the DNA which is a region on the DNA, which is also designated as ISRE and binding of this STAT and the IRF in this region. So this STAT dimer along with the IRF they bind to this region.

And that leads to the over expression or production of different downstream proteins. And that proteins or those proteins are the mediators of the action. So what are those produced? They are usually the genes that produces the proteins. They are known as the interferon stimulated genes or the ISG. So they usually leads to the upregulation of interferon stimulated genes.

So these are the ISGs or the interferon stimulated genes that are upregulated when type I and type III interferon binding occurs and there is JAK STAT signaling. Similar to this in case of the type II or the gamma, interferon gamma also there are elements or response elements in the DNA where this STAT dimer goes and binds.

The STAT dimer goes and binds and this element in this case is known as the gamma interferon activation site or the GAS, the gamma interferon activation site or the GAS. So now once there is a type II interferon or gamma interferon binding, there is this kind of receptor tetramerization leading to the JAK STAT signaling.

And this STAT, the dimer of the STAT the STAT dimer then goes and binds to the DNA elements which are also known as the gamma interferon activation site or the GAS and that also leads to production of different ISGs. So the main mediators of function are basically the ISG. So the idea is very clear.

The idea is when there is interferon binding to an interferon receptor that leads to activation of the JAK STAT pathway leading to the STAT dimerization. And STAT as it is an activator of transcription, so it is a transcription activator.

So it goes and binds to the DNA element, activates the transcription or enhances the gene expression of certain genes, which are the ISGs or the interferon stimulated genes that produces some protein molecules or proteins downstream, which mediates

the function. Now type I and type III interferons are primarily responsible for antiviral activity. And that is why interferons are very important.

You must be knowing that sudden interferons primarily interferon alpha, so recombinant interferons are nowadays used in certain viral infections, so to treat viral infections like for example hepatitis. So interferons are being used for treatment as well. So the interferons has a very high antiviral activity and antiviral activity means the interferon itself is not antiviral.

So very clear, interferon itself does not have that antiviral activity. But it can induce a signaling downstream of binding to the interferon receptors, that can lead to some expression of genes and expression of proteins that has antiviral activities. And this antiviral activity is primarily promoted by type I and type III interferons. The type II or the gamma interferons are primarily responsible for antimycobacterial or any other non-viral infections, any bacterial infections.

So they impart usually antimycobacterial immunity and also against many other pathogens, but not viruses. So the main antiviral activity resides in type I and type III interferons and primarily interferon alpha. Interferon alpha, which is a type I interferon. So now the question is, how this antiviral activity is being mediated by this interferon. So what is the mechanism of action?

So this is the first the upstream process, that is interferon binding to the receptor and then activating the genes or activating the overexpression of the genes that are actually responsible for the antiviral activity or the ISGs that is the interferon stimulated genes. Now what are these ISGs? What are the different types of ISGs that actually mediate the antiviral activity?

So if we look into the starting from here again, so if we look into the ISRE leading to the production of or of the ISG or the interferon stimulated genes. And this interferon stimulated genes can actually be classified in many, there are many interferon stimulated genes but the most well studied, so we will be discussing about the most well studied of this interferon stimulated genes.

What are the most well studied processes that actually impact the antiviral activity? So one are the Mx GTPases, the Mx GTPases. A second class of proteins are the oligoadenylate synthetase or OAS and RNase L mediated antiviral action. And a third way of antiviral activity is mediated by protein kinase R or PKR. So what these three different things they mean actually?

So MX GTPases the OAS, and RNase L, it is a combined effect of the OAS and the RNase L and the protein kinase R. So these are the three major, there are many other ISGs. So these are the ISGs, the interferon stimulated genes whose expression is increased. They are also expressed normally, but they are not overexpressed. They are not over produced in the cell.

Whenever there is a viral infection, there is secretion of the interferons or production of the interferons increase in the secretion of the interferons different cells of the immune system as we have learned from our previous classes, the different cells of the immune system they start secreting cytokines. And interferons being one of those cytokines whenever there is a viral attack, the interferon secretion increases.

And when this interferon secretion increases, it leads to the overproduction of these ISGs, the downstream ISGs by this JAK STAT mediated mechanism. Now what are the three different these are the three ISGs that I have written here are the Mx GTPase, the OAS and the RNase L and the protein kinase R. These are the three different GTPases, three different ISGs or the interferon stimulated genes that are responsible for imparting the antiviral activity.

Now let us see how they impart the antiviral activity. The Mx GTPases when they are overproduced they start to oligomerize. So there is an oligomerization process, oligomerization of the Mx proteins or the MX GTPases and this oligomerization of the Mx proteins the Mx protein oligomers that are produced, they can actually bind to the viral nucleocapsid.

They can bind to the viral nucleocapsids and by that they can kind of sequester the virus and trap the virus. So it can trap the virus by binding to the viral nucleocapsids. It traps and degrades the virus, okay. So these are, this is mainly the action shown by

the Mx GTPases. So Mx GTPases are overproduced in presence of interferons when there is interferon signaling, this Mx GTPases they are overproduced.

And once they are overproduced, they start to oligomerize. Once they oligomerize then this oligomers, the Mx GTPase oligomers, they can go and bind to the nucleocapsid of the virus and then they can trap the virus or kind of sequester the virus and degrades it. It then degrades. So this is one of the processes by which the ISGs can mediate the antiviral activity. A second way of doing it is by the oligoadenylate synthase.

Now what is this oligoadenylate synthase? So oligoadenylate synthase is a particular class of proteins, which can actually make 2 prime to 5 prime phosphodiester linkages. Normally, in our nucleic acids in DNA, we find a 3 to 5 prime phosphodiester linkage. But these oligoadenylate synthase, they can actually lead to or they induces formation of 2 prime, 5 prime oligoadenylates.

So they just join this ATP molecules and produces in the 2 to 5 prime joining, they can produce oligoadenylates. And this oligoadenylate once they are formed, it can activate a specialized class of RNAs. Now what happens is when interferon signaling occurs, interferon type I interferons or type III interferons, they bind and lead to the overexpression of this ISG because OAS is one of those ISGs.

So when there is an overexpression of the oligoadenylate synthase, the oligoadenylate synthase usually is present as a monomer. It is a monomer. It is usually a monomer. But this monomer is in, which is an inactive form. Now when this when there is a viral attack, so it interacts with the virus. It can directly interact with the virus primarily with the genetic material of the virus. So with the viral RNA, for example.

So it interacts with the viral RNA and it can oligomerize. So this OAS then can form a tetramer. So now this tetramer is the active form. So this oligoadenylate synthase when it is produced upon interferon signaling, it usually exist as a monomer. When it can interact, this monomers of the oligoadenylate synthase interacts with the viral RNA, it starts to tetramerize. Now it forms tetramers.

Now this tetramers when they are formed, this tetramers they can actually induce the synthesis of 2 prime, 5 prime oligoadenylate. So this 2 prime, 5 prime oligoadenylates are being now produced after tetramerization. So now this is the tetramer. So this monomer is the inactive form and this tetramer is the active form. So this of the OAS. So this is the OAS. We have not spoken about the RNase L yet.

So now this 2 prime, 5 prime oligoadenylate that is being synthesized by the tetramer tetrameric OAS, this can interact with RNase L. RNase L which is also normally produced in the cell in very minimal amount in constitutive amount is being overexpressed in response to interferon signaling. And that is why it is also one of those ISGs. So it is also an interferon stimulated gene. So RNase L is also overproduced.

Now this RNase L when binds to this oligoadenylate synthase, it also dimerizes. So RNase L normally remains as a monomer. So it is normally a monomer. When there is binding of this oligoadenylate synthase sorry, 2 prime, 5 prime oligoadenylates when they bind to this RNase L that leads to dimerization. So RNase L dimers are formed and this RNase L dimers these are the active form.

The RNase L monomer is the inactive form. The RNase L dimer is the active form. And this RNase L dimer now can cleave or chop off the viral RNA. So it cleaves the viral RNA, clear? So it can now cleave the viral RNA. A third pathway is about the protein kinase R or the PKR. And these are also induced by the interferons. So this protein kinase R is also produced in response to interferon binding.

And once they are induced by interferons, these PKR monomers are synthesized. And again, very similar to the OAS, the PKR monomers, they are also activated by, activated by the viral RNA. They can also bind to the viral RNA. And when they bind to the viral RNA, the PK monomer then they are phosphorylated. And this phosphorylated, so this monomers is the inactive form.

When they binds to the viral RNA, the PKR phosphorylation occurs. And that leads to the PKR dimer formation. Now they becomes PKR dimers, the protein kinase R dimerizes. So there is a dimerization. And now it becomes active. So now this PKR

dimer is an active form of the PKR and as the name suggests it is a protein kinase. So it can also phosphorylate other proteins.

So it starts to, its target protein is the initiation factor or the in the translation eukaryotic initiation factor, it starts to phosphorylate the eIF-2 alpha which is a translation initiation factor and thereby it stops the process of or blocks strand inhibits translation. So and so in turn it kills the virus.

So if we look into this three pathways that are being induced by this ISGs primarily the interferons, so whenever there is an interferon signaling, the ISREs there is binding of this the STAT and the dimerization of the STAT this portion, so starting from here again, so there is dimerization of the STAT and leading to binding to the ISREs, leading to the induction of these genes, the interferon stimulated genes or the ISGs.

Now this ISGs, the major three ISGs are this Mx GTPases, the OAS and the RNase L and the protein kinase R. As I described, this Mx GTPases they are produced in huge amount and they start oligomerization. Once they start to oligomerize, they can react or they can interact with the nucleocapsid of the virus. They binds to the nucleocapsid of the virus and by that they can trap the virus and degrade it. So this is one process.

The OAS or the oligoadenylate synthase normally is produced as inactive monomers. This is a inactive monomer. Now this monomer can interact with the viruses and primarily with the viral RNA. So they interacts with the viral RNA and it forms a tetramer. Now this tetramer is the active form. Now once it forms a tetramer it starts to synthesize this 2 prime, 5 prime oligoadenylate.

And this 2 prime, 5 prime oligoadenylate in turn activates the RNase L and helps in RNase L dimerization. So this 2 prime, 5 prime adenylate, 2 prime, 5 prime oligoadenylate is required for activation and dimerization of the RNase L, okay. So RNase L is activated and it is it dimerizes. Now this dimeric RNase L can cleave the viral RNA as well as the RNA of the cell, so the infected cell.

So and then it kills or destroys the virus. And then one of the third process is the protein kinase R or the PKR. So the PKR is again synthesized as monomer as a monomeric unit and it is also it can interact with the viral RNA. So once it interacts with the viral RNA, it is activated and PKR once activated this PKR monomers they get phosphorylated. So this PKR this protein kinase R monomers they are phosphorylated.

And this phosphorylated monomers, they can now form PKR dimers. And the PKR dimers is the active form. So this PKR monomers are the inactive form and this is the active form. So this is the active form and this is the inactive form. So now this PKR dimers they become active and they are the active form. So they become active and they are the active form of the PKR or the protein kinase R.

And one of the target proteins of the PKR is the eIF-2 alpha. So initiation factor in the translation. And it can phosphorylate the eIF-2 alpha or this initiation factor and by that it can inhibit the translation and thereby it can also inhibit the viruses or destroys the viruses.

So overall this interferons as we understand from here, the interferons they mediate their actions by binding to interferon receptors leading to, when they bind to the interferon receptors leading to downstream signaling that leads to the overexpression of the ISGs. And the ISGs in turn, they have they mediate the antiviral activity.

So we primarily discussed about the antiviral activity of the interferons, which is mainly mediated by type I and type III interferons. We have not discussed about the type II interferons. Type II interferons are primarily responsible for imparting antimycobacterial immunity and other bacterial immunity as well. They are not related to antiviral activity.

So this is a brief mechanism of antiviral activity mediated by the interferons. And that is all for today's this class. And thank you very much.