Host-Pathogen Interaction (Immunology)
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Lecture - 59
Innate Immunity During Virus Infection

So, in previous session we have learned a lot about the PRR mediated antiviral immune response which is mainly the interferon dependent. Now in this session I will discuss about the interferon independent processes by which the viral replication is checked. In fact, in this session, I am going to talk a few of my work my own work my own laboratory work which very clearly demonstrate that some of this post transcriptional regulators they play a very important role in checking the viral replication. Let us begin.

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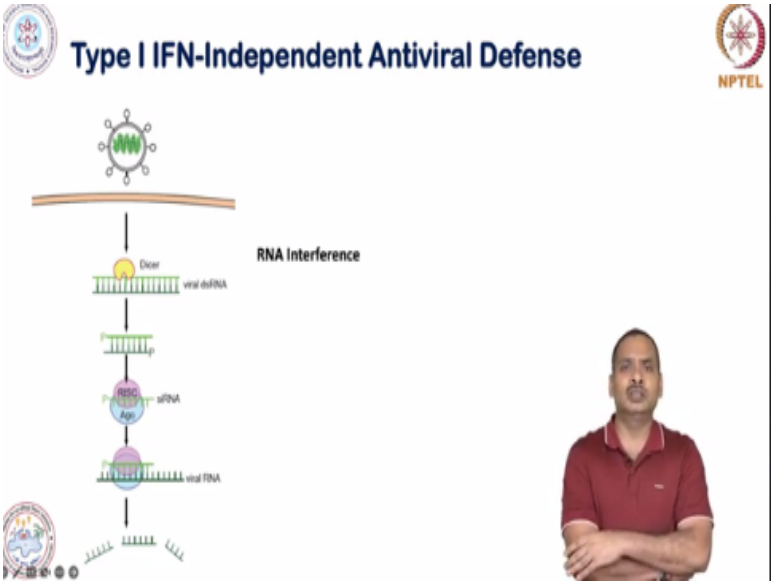

So, here this is a very simple schematic and this is true for many other organism. There are some this is some small interference RNA which plays a very important role in defense against the viruses. Basically, there is a small interference RNA they interact with the genome of virus particularly RNA viruses and then this will trigger basically the cleavage of the target RNA. So, this is one way and this thing I will elaborate more in subsequent slides.

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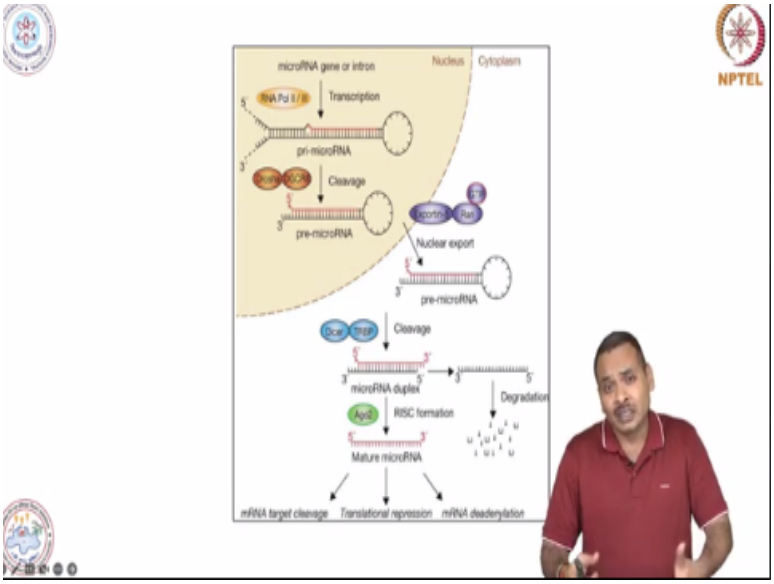

In mammals also there is or in human there is also expression of these small RNA which we call it as a micro-RNA. Here you can see the biogenesis pathway and towards end there will be a generation of mature micro-RNA and this mature micro–RNA is basically the key function of this micro–RNA is to regulate the gene expression at transcript level. So, these micro-RNA basically interact with the transcript gene transcript or mRNA.

And as you can see that they basically play a very important role in reducing the expression of this transcript or mRNA and this is basically achieved by one protein complex which we call it as a RISC complex. This RISC is a RISC it is a RNA induced silencing complex and this RISC complex is basically consists of several protein including one very important protein known as agonate 2.

So, this agonate 2 basically facilitate the bridging or binding of a transcript with the micro-RNA. And then once this RISC complex is formed then there will be a there could be a several fate. Here you can see that the target mRNA can be cleaved. Another is there could be a translational repression there. So, they will be so if there is a actively transcribing translating mRNA then they will kind of a bump. The translational machinery cannot move if it is there.

This can also trigger the mRNA de-adenylation and that de adenylation basically make the mRNA unstable. So, these are the ways by which these micro-RNA act in the cell and they are

playing very important role in regulation at transcriptional level or post transcriptional their post transcriptional regulator and they are kind of fine tuners for the expression of a particular gene. And these micro-RNA originally discovered as a regulator for the gene but we have found out that besides regulating the transcripts they are playing very important role in virus defense.

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Here you can see this work. Here you can see that the RNA from RNA virus. This basically sensed by the RIG I as you have studied a lot which is translated from the RIG I transcript this RIG I basically senses this RNA molecule viral RNA molecule and this RIG I protein is synthesized from transcript of RIG I. And upon sensing of this viral RNA by RIG I it will induce the pro-inflammatory cytokine and type 1 interferon that you know very well.

Here we found out that this viral RNA when it goes inside the cell it induces the expression of some micro-RNA. And this micro–RNA is there are several micro-RNA among these micro-RNA one micro–RNA is there known as micro-RNA 485.

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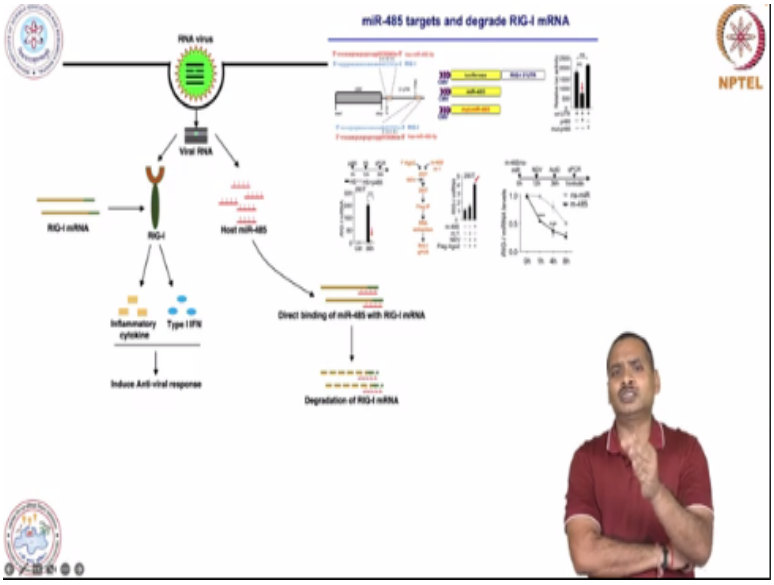

And this micro-RNA 485 basically plays a very important role in regulating the transcript of RIG I. Here you can see in this schematic this micro-RNA can interact with the RIG I. Here you can see the structure of gene in these experimental results. So, this micro-RNA can bind to the two regions. Here one more important formation I missed in previous slide. Generally, these micro-RNA bind with three prime UTR of the gene in general.

In rare cases this micro-RNA can bind to the five prime UTR but in general it is binding to the three prime UTR and then that by binding it regulate the translation of the transcript. Let us come back. So, here you can see that this micro-RNA 485 can bind with the three prime UTR and then this is reducing the expression of transcript. So, when this is the situation, in this situation if you infect the cells with viruses so what will happen? The simple thing is that there will be a more load of viruses.

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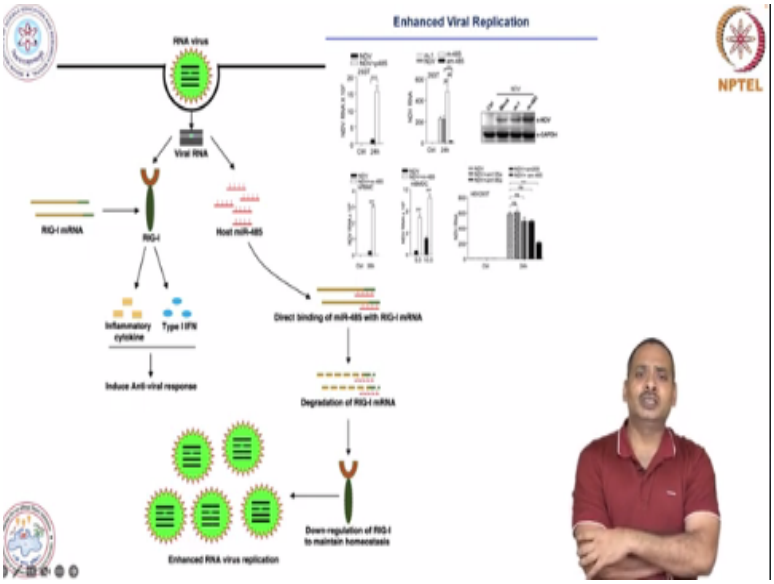

So, because the expression of this RIG I is reduced. So, expression of RIG I is reduced it mean there will be a reduction in production of antiviral cytokine that is inflammatory cytokine as well as type 1 interferon. So, if this down regulation is there then there will be a more increase in amount of viruses in the cell. If you in this scenario if you infect the cells with viruses then there will be a increase in amount of viruses as you can see in this experimental data.

When we have introduced this micro-RNA here P-8485 is just a plasmid which is expressing the micro-RNA 485 inside the cell. So, when we introduce this thing then there is an increase in viral load. Here you can see that NDV RNA this is we have measured the virus specific RNA so which is very much higher. So, this slide basically show various kind of experiment and here the overall conclusion from this experiment is there will be enhance in the viral replication.

So, we perform this experiment with different viruses. Here you can see that there is a NDV virus result we have performed with many other viruses. We also perform this experiment with influenza virus.

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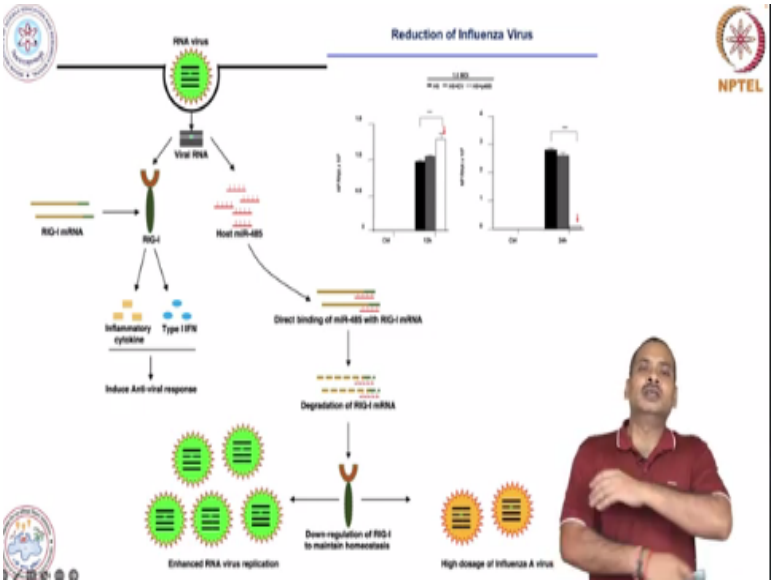

So, when we have performed this experiment with influenza virus very surprisingly it is a serendipitous discovery. It is a discovery by chance, we found out that this when we infected the cells with influenza virus which contained the micro-RNA 485 initially there is an increase in viral load the cell look not so healthy initially and after some time the cell looks very healthy. So, here you can see that at early time point there is an increase in viral load, cell are unhealthy.

But we just perform this experiment at a higher time point then we found out that cells are much more healthy, they become a normal. So, in this scenario we wanted to know why it is there. Of course, we are performing this experiment. So, we doubted our experiment maybe something we have done wrong. We figured out that there is nothing wrong but this is some phenomena. So, in order to understand why the cell, become healthy we have performed more experiment and try to find out what is the molecular mechanism.

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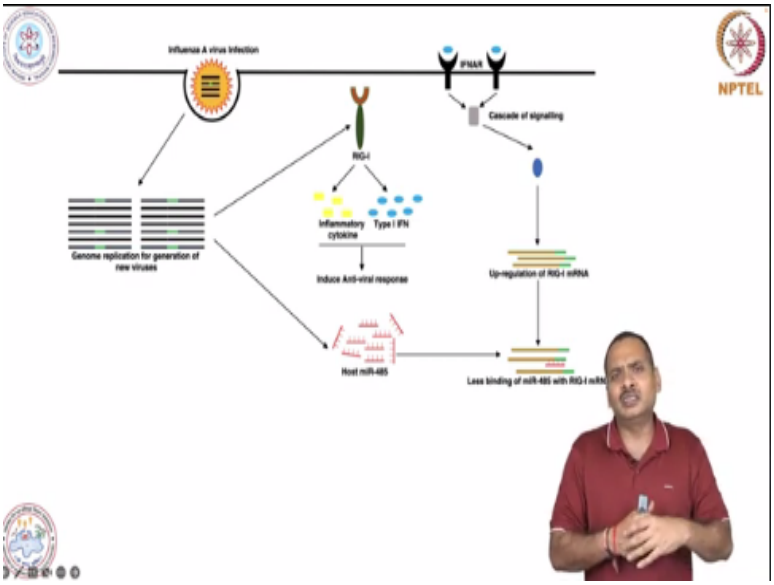

So, here again you can see that when there is influenza virus infection there will be the influenza virus genome will be a lot in the cell. So, here just I want to say that I will talk more about the influenza virus when we will take up the influenza virus. So, in this course I am going to discuss about the influenza virus infection and all those things. So, let us come back. So, what happened when this influenza virus infect the cell then there will be a viral replication.

Lot of influenza genome will be generated inside the cell and this will be sensed by the RIG I and then there will be induction of antiviral response. This antiviral response particularly the production of type 1 interferon this will activate another set of signalling which we call it as a JAK STAT pathway which will the outcome of this JAK STAT pathway is to make more type 1 interferon. A lot of type 1 interferon and the RIG I is a interferon inducible gene.

This is very well known. It is a like a you have seen RANTES and IP10. So, RIG I is also one of this molecule it is an interferon inducible gene. So, that will induce lot of transcripts of RIG I inside the cell. And here you can see that when this virus will infect there will be a viral genome inside the cell and this will also induce the synthesis of micro-RNA 485. I have showed you in the previous slide.

So, this micro-RNA 485 basically interact with the transcript of RIG I. In order to reduce the expression of RIG I, try to understand any immune response should be also damped after some

time. If it will be not damp then that will result to the autoimmune disease, too much immune response is also not good. So, this micro-RNA 485 is basically playing a very important role in maintaining homeostasis inside the cell.

So, that is why this is binding with the transcript of RIG I and then because when the virus is cleared then there is no need of RIG I. So, this will basically bind with this transcript of RIG I and then it will damp.

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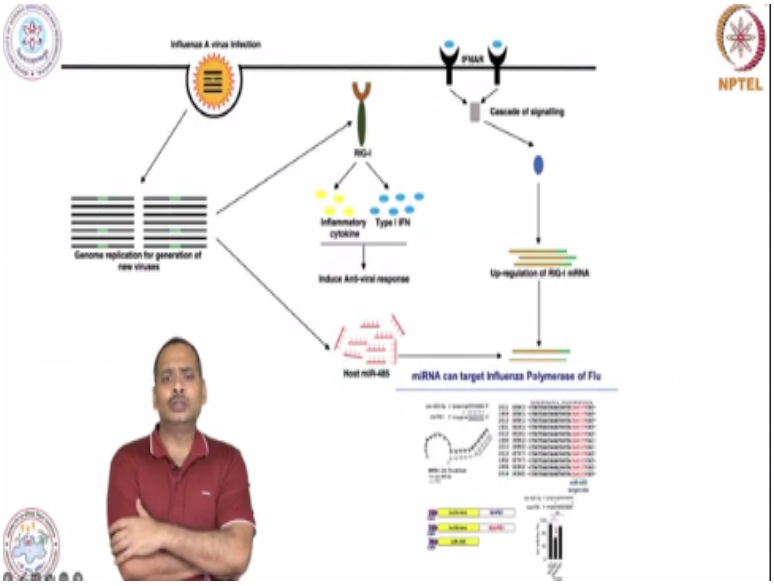

So, in order to find out why the viral load is reduced after influenza virus infection we have looked at the genome of this influenza virus. And we found out that this micro-RNA 485 can bind with one of very important gene of influenza virus which is needed in order to make a more copies of genome, it is basically RNA polymerase. So, this RNA polymerase is consists of three subunit and this micro RNA 485 basically interacting with one of this subunit.

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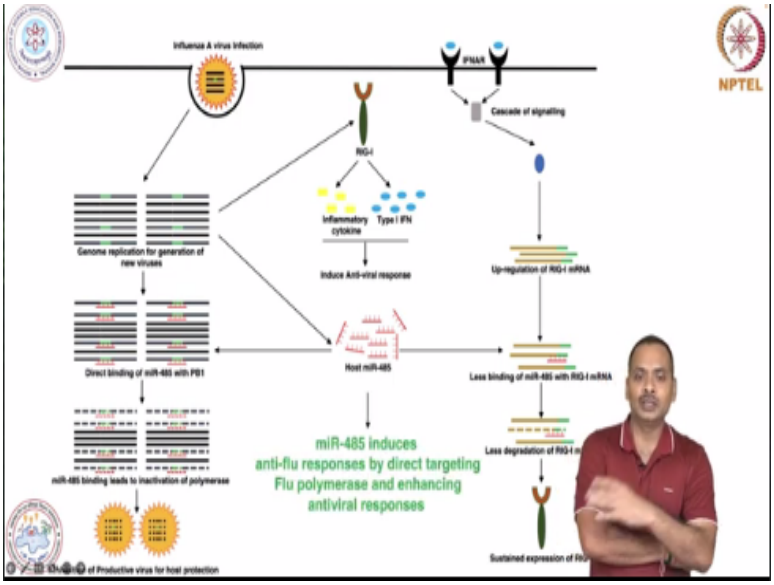

So, once it is interacting then it is degradating as you can see in this schematic. Here it is interacting with the genome of influenza virus and then it is degradating. So, we have proved this experiment by creating the mutant influenza virus. In the lab we have created the influenza virus there is a system known as reverse genetic system. So, if you use this reverse genetic system, you can create the influenza virus in the laboratory.

So, we have created and we have mutated that site and then we have concluded that this micro-RNA 485 basically targets the influenza virus genome and it is reducing. That is why after some time the influenza virus is basically since it is a targeted the amount of influenza virus in the cell is reduced. So, what is happening overall? So, once this micro–RNA is binding with the genome of influenza virus then it is less available for RIG I transcript.

And when it is less available for RIG I transcript then what will happen? There is a more RIG I expression. So, basically if you see carefully or observe all these phenomena carefully you can find out that this micro-RNA 485 this is acting as a double-edged sword against influenza virus infection. One is they are targeting the genome of influenza virus and checking the replication, another is this micro–RNA is less in the cell because most of micro–RNA is targeting the influenza virus genome.

So, then what is happening? The transcript of RIG I will be more available for translation. And if there will be a more availability for translation then what will happen? It will be basically there will be a more expression of RIG I and more sensing of this viral RNA and then there will be a more type 1 interferon. So, here you can see that the sustained expression there will be a sustained expression of RIG I which is currently needed during the influenza virus infection.

So, in that way this micro-RNA 485 act as a double-edged sword. So, we have proved this thing by creating a variety of mutant influenza virus and finally we successfully showed these phenomena. So, here you can see that this micro-RNA 485 is a kind of interferon independent. It is induced by the viral genome and it is targeting the influenza virus genome and then it is checking the influenza virus.

So, here overall conclusion is that this micro-RNA 485 induces anti-flue response by direct targeting the flu polymerase which is needed for the replication of virus and enhancing the antiviral responses because of sustained expression of RIG I.

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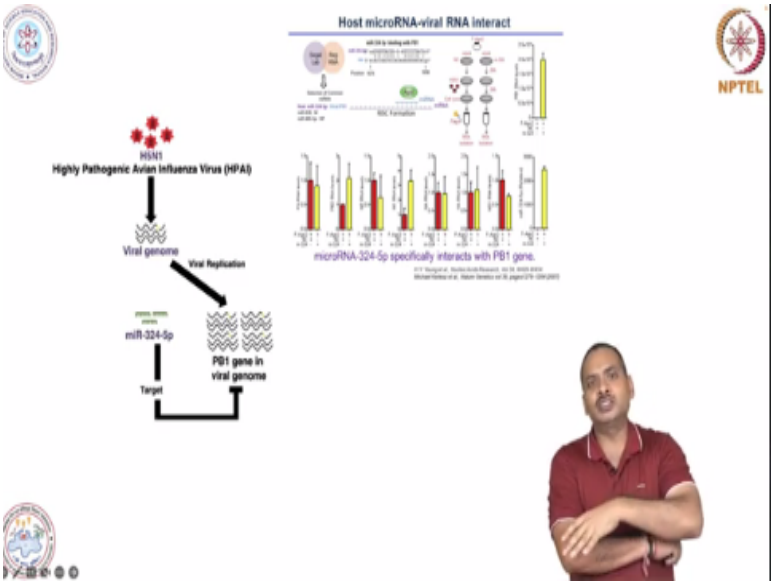

There is one more example. So, in cell there is a one micro-RNA that is micro-RNA 324. So, this micro-RNA 324 is present in the cell and when there is a virus infection this virus down regulate the expression of this micro RNA 324 and there is a reason, why? Because this 324 can target the again polymerase of influenza virus. Since it is targeting then this will reduce the viral load.

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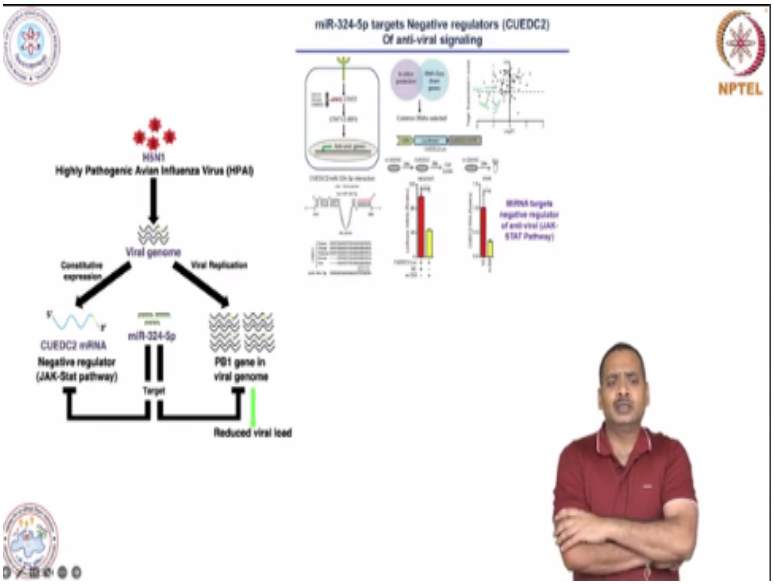

And this micro-RNA can also target the negative regulator of JAK STAT pathway. I have told you JAK STAT pathway is basically inducing more type 1 interferon. So, since it is targeting the negative regulator so this micro-RNA again act as a against the influenza virus or other viruses. How? If it is targeting the negative regulator then there will be a more production of type 1 interferon and type 1 interferon is antiviral.

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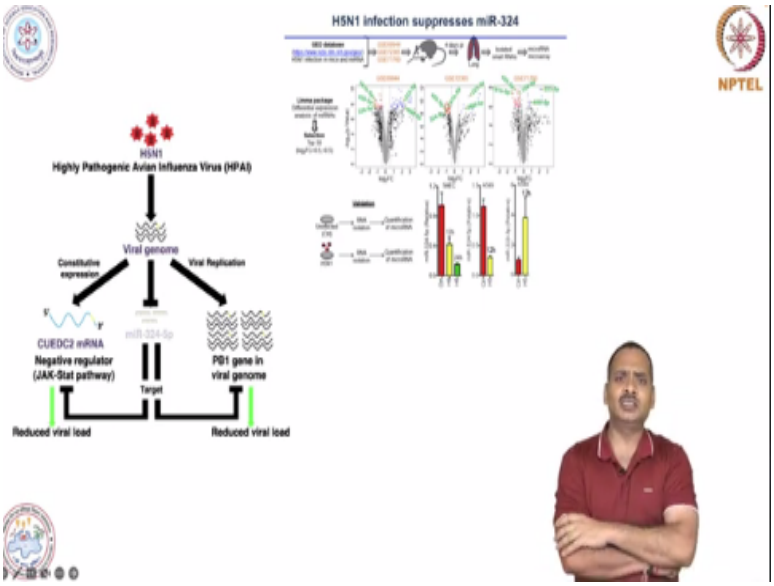

So, this is the situation and here we showed that when there is an influenza virus infection in the cell then it reduces this micro-RNA 324.

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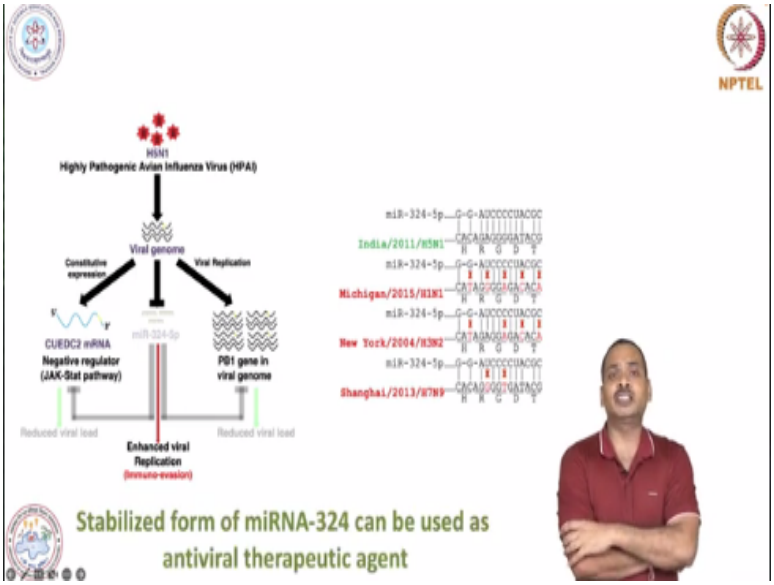

It is a basically immune evasion mechanism because this micro–RNA is targeting the influenza virus and then it is basically stopping the replication of influenza virus. So, virus what it is doing? It is evading the immunity; they reduce the expression of this micro-RNA. In addition, we found out that some of the recent strains of influenza virus they are changing their sequence in the genome where it is binding.

So, if there is a change in sequence then this micro-RNA cannot bind and another very important lesson is that in addition of changing, they are maintaining the amino acid sequence. You know one amino acid can be encoded by various codons. So, in that way they are evading the immunity. So, if we use this micro-RNA 324 the stabilized form this can be act as a antiviral and this is also the interferon independent.

So, this is I have talked about the interferon independent processes or molecular mechanism by which the viral replication is checked. In next session I will talk about what are the viral factors which is used by the virus in order to evade the immunity. So, with this I will stop here, thank you.