RNA Biology
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Lecture - 46
Dosage Compensation, Xist and ncRNA in Imprinting: shRNAs and Gene Expression

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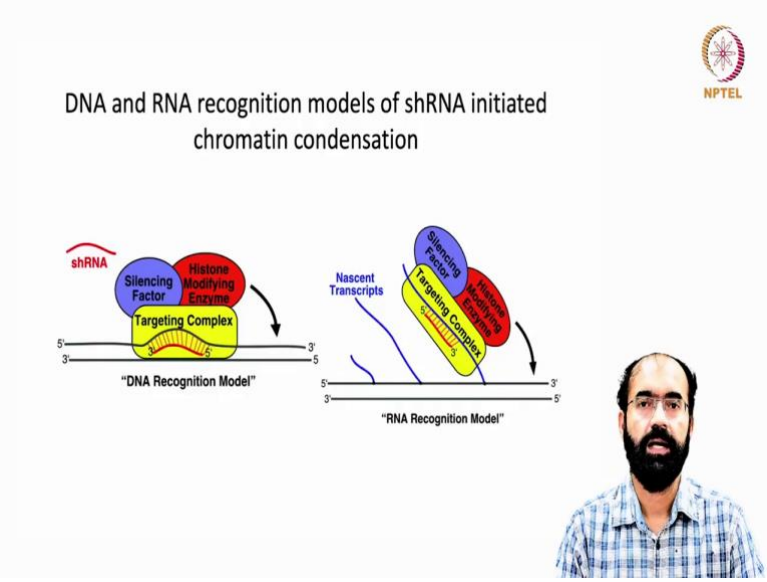

Hello everyone, welcome back to another session of RNA Biology. So, we were learning the importance of shRNA in regulating the genomic imprinting and there are two models. One is DNA recognition model and RNA recognition model. In both the models it is following similar pattern. Except that in DNA recognition model there is no need of gene expression. It can be imprinted because of an shRNA coming from elsewhere binding onto one of this DNA strands and triggering the silencing event.

Whereas in RNA recognition model this gene has to be expressed just like Xist has to be expressed. This gene has to be expressed for a while and then this will be triggered by the shRNA binding and it will lead to the silencing through the silencing complex as it happens. So, one is acting onto the DNA, the other is acting onto the RNA. So, this is the only difference otherwise it remains more or less the same.

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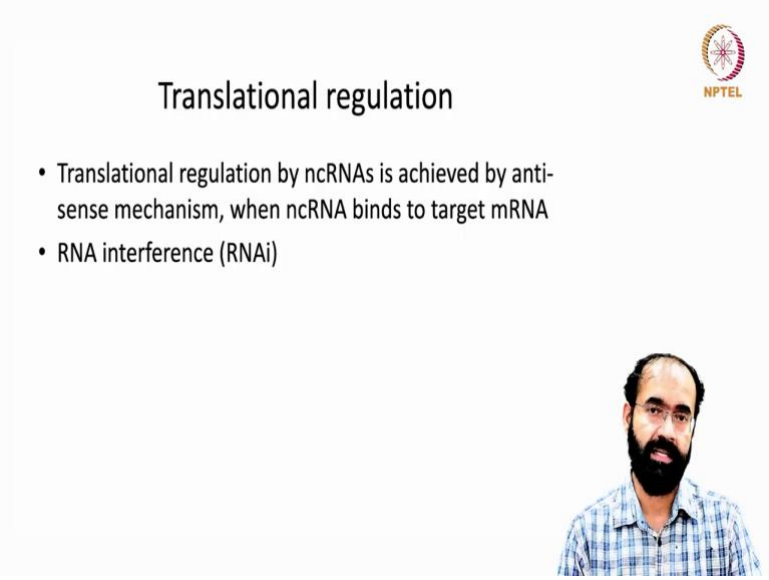

Now, let us see the translational regulation. So, many non-coding RNAs they have the translational regulations. So, this translational regulation by non-coding RNA is achieved by antisense mechanism mainly when the non-coding RNA binds to the target mRNA. So, this is a kind of RNA interference, RNAi. So, RNAi can happen mainly two types. One is a full length of RNA pairs with a full length of antisense RNA. This is one way of RNAi.

We have seen with the xist and T6 and we also saw several other examples where the regulation of some genes in young cells and old cells how it is done. So, RNA interference can happen even if it is pairing only a small length or small stretch. This also can happen and normally the microRNA and siRNA all done through this small stretch because they are of only around 22 nucleotides in length microRNA or siRNAs.

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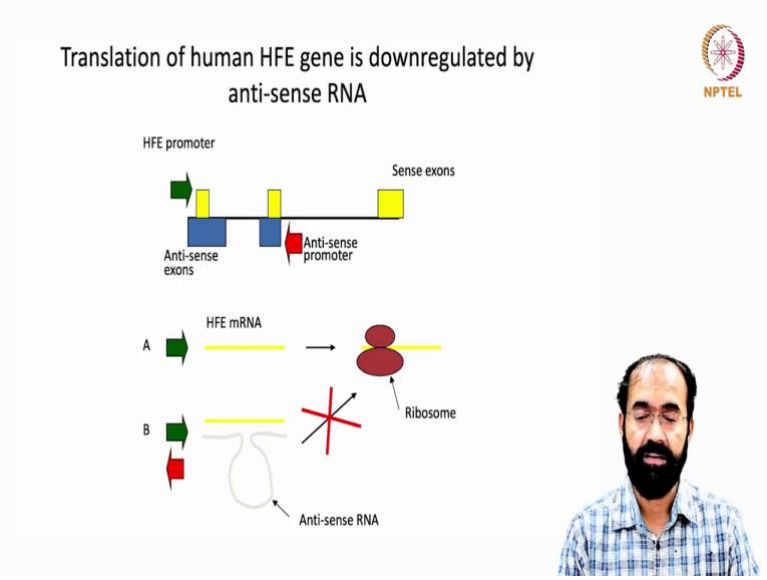

So, let us see some examples how this translation of human HFE gene is down-regulated by antisense RNA. So, HFE is basically for iron utilization. Ok Fe stands for iron and high ion. So, HFE gene can have a its HFE gene specific promoter and it has yellow color boxes are exons and you also have antisense strand just like xist and T6 we have been talking about.

In the antisense promoter you can have the production of this antisense RNA. So, HFE is the sense gene and it is having the exons responsible for HFE protein production and these exons are properly spliced later and the RNA can be found. But on the other hand, the other strand of this DNA can have a antisense promoter which can make an anti-HFE RNA that is an RNA complementary to the HFE RNA. And this does not have all the three exons, but it do have somewhat complementary region to two exons much larger.

So, this yellow box is smaller, but this blue box is longer and this yellow box is smaller and this second blue box is longer. So, this is the first one, this is the second one. Here this is the first, second and the third. So, for antisense this is the first, this is the second. So, in either case it has sufficient region that can have an overlapping region to that of the earlier that is the HFE RNA. So, HFE mRNA is formed and it can participate in the protein translation.

However, if the antisense promoter is turned on and if antisense RNA came out then what will happen? The portions of this HFE mRNA will be now paired by

complementary region. Throughout it cannot pair because we know this area will not find it pairing, this area it will not find pairing.

So, this area it will pair and same with this area it will pair. So, that is what you are saying this yellow region will find it complementary sequence to the antisense sequence and that will cause the degradation of this HFE mRNA and there is no protein, HFE protein will not be produced.

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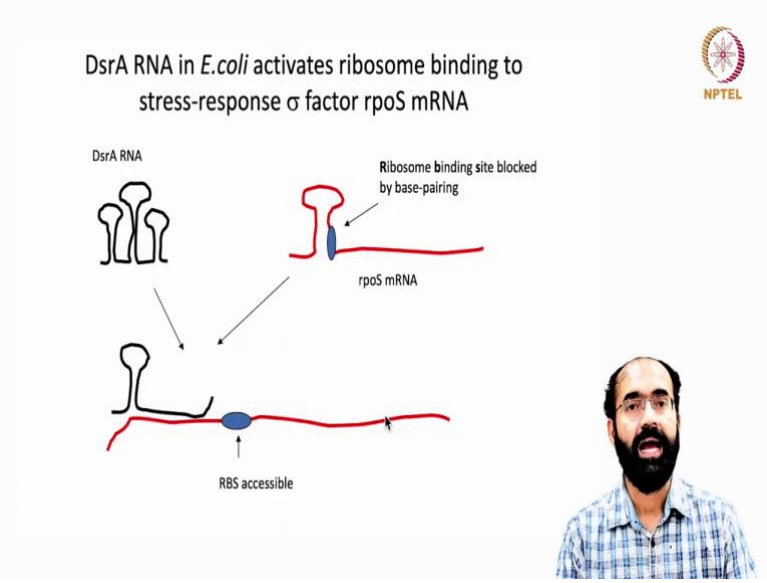

And similarly in E. coli also somewhat similar mechanism is been followed that is DsrA RNA in E. coli that can activate the ribosome binding to the stress response sigma factor rpoS mRNA. Let us see how is it doing?

So, DsrA RNA is here and this is somewhat similar to the shRNA what we have been discussing or the antisense RNA we have been discussing. So, this is the RNA that is meant for rpoS RNA. rpoS RNA is basically a sigma factor function that is meant for like we have seen several sigma factors when we studied bacterial gene expression.

So, interesting thing is this rpoS RNA have got a ribosome binding site that can be blocked by base pairing. So, you have two ways of controlling it. One way if this DsrA RNA is present then this RBS that is ribosome binding site is accessible and the protein can be translated. However, if DsrA RNA is not there it is not available what will

happen? This ribosome binding site is masked because of this stem loop secondary structure.

So, rpoS RNA which is a sigma factor RNA if it is present does not mean it will get translated. It by default is kept suppressed it is not getting translated, but it will wait for the secondary regulator that is an antisense RNA DsrA RNA. If DsrA RNA comes into picture.

Then this rpoS RNA pairs with it and it blocks this secondary structure formation because it is now opened up it is pairing with this DsrA RNA which will expose the ribosome binding site and this rpoS protein can be formed and it can drive the expression of a bunch of other genes

Because sigma factor is basically acting like a supervisor directing the RNA polymerase 2. You have studied the tetrameric core and the hollow enzyme assembly etcetera. So, this RNA polymerase can easily start functioning if this rpoS sigma factor is available not all the genes some specific genes.

So, in the previous example we saw that the antisense RNA can prevent it can prevent the HFE gene expression if it is present whereas, in this example we are seeing the rpoS RNA will be allowed to produce the protein in presence of a non-coding RNA. So, both examples we have sometimes the RNAi will prevent the gene expression whereas, in this case of E. Coli it is facilitating the gene expression or the protein production from a given RNA.

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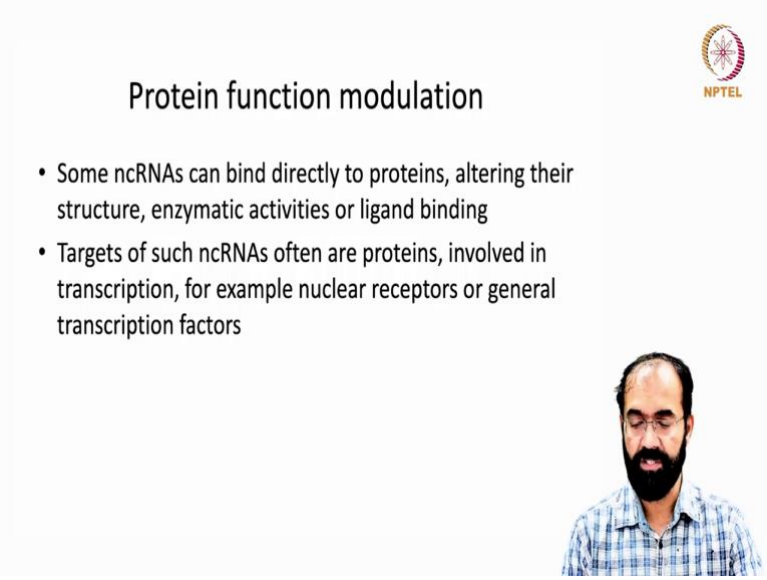

And non-coding RNA can also help in the modulation of protein function. Some Non-non-coding RNA can bind directly to proteins altering their structure enzymatic activity or ligand binding because that is another function of the non-coding RNA that they can modulate means, tweaking it just like you can see like if you are; if you have talent of you are a sprinter you have the inborn talent of running fast.

So, but if you get into a good coach. So, he will give you proper exercise that can accelerate the strength or increase the muscle that is required for running. So, you already have those muscles perfect everything is fine, but you also have maybe you are a little bit overweight or you have more body mass etcetera.

So, he will tune you such a way that your muscles mainly required for running purpose. The same muscles may not be helpful for playing a badminton or same muscles may not be helpful for playing football.

But if you are a sprinter certain muscles need to be strengthened although in playing badminton also you need to run while playing football also you need to run, but they are different running football you do not run in a straight line you run in you know zigzag motions, you have to suddenly turn twist your body, but if you are a sprinter you run in straight line.

So, we cannot generalize saying that you know running all are not running that is called modulation what I meant here is depending upon which coach is handling you he will help you in strengthening certain muscles in your body and you will excel. So, modulation is somewhat similar that it is not altering the property the fundamental property of that protein, but it will facilitate doing the function in a given fashion so, that altering their structure enzymatic activity or even the ligand binding.

So, some targets such as the non-coding RNAs often are proteins and involved in the transcription for example, the nuclear receptors or general transcription factors. So, many non-coding RNA targets those proteins that are mainly doing the job of transcription and it has got an advantage because transcription is a general event.

Like if you want to turn off all the lights in your house you will go to main switch instead of going to 10 rooms and switching off all the lights you simply go to the main switch and turn it off all lights will be turned off. Some houses you also will have a master switch where all are connected to the light you turn it on or off that particular switch can control it.

So, transcription factors are something like that because they are important in a bunch of gene expression events like we studied we mentioned about pluripotency factors four of them can convert a skin cell into stem cell. So, that does not mean that each of these proteins are doing only one job. These proteins turn on a group of genes a large number of genes and that large number of genes and their products are the one which converts the skin cell into stem cell.

We cannot say that oh difference between a skin cell and the stem cell is only four genes. No. Because these four genes are very important in turning on a bunch of genes that is why they become pluripotency factors although they are transcription factors. Similarly, the noncoding RNAs can influence the proteins of some big importance or a wider importance. So, that is why it becomes so, important that they will be able to handle the whole process in a effective manner.

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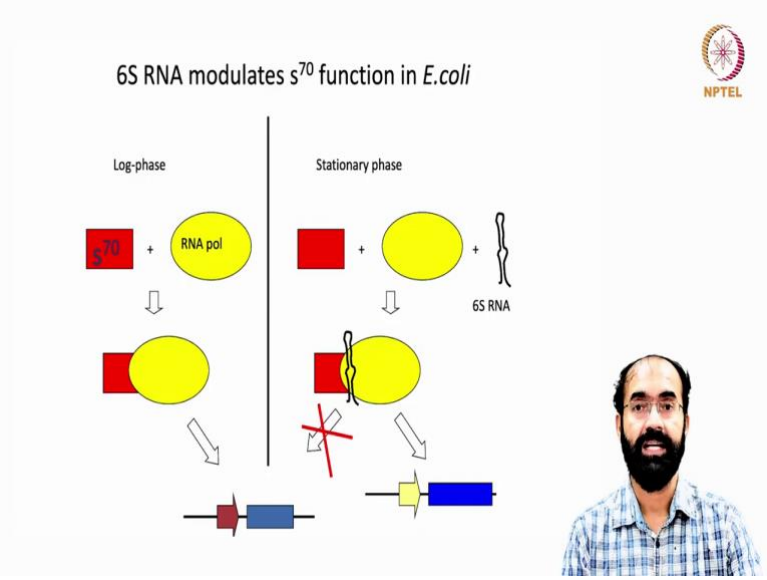

So, let us see an example. 6s RNA that can modulate sigma 70 function in E. Coli. Let us see a situation normally we know bacterial grows in three phases. One is a lag phase say you took a medium and inoculated bacteria. The first stage is lag phase then it has got a log phase and then it has got a stationary phase. Normally you measure the bacterial density in absorbance at 600 nanometer in a spectrophotometer.

So, if the optical density is below 200 you are normally referred to as lag phase. 200 to 600 is considered the 200 to 700 roughly is the log phase 700 to 1 that is the 1000 that is the normal 0.6 you can refer whichever way you want to. So, normally the lag phase followed by log phase and followed by stationary phase. So, a bacteria is in its perfect growth when it is in the log phase. Lag phase it is increasing in number log phase it is performing excellent.

Once the nutrition becomes depleted it will enter into the stationary phase. So, this is a typical and it will look like a s sigmoidal curve that is the typical s-shaped curve and that is what we call it as bacterial growth curve. So, in log phase you have sigma 70 and RNA polymerase turns on a bunch of genes for the survival and maintenance of the organism and utilizing the nutrition.

However, once it is stationary phase you do not want such kind of level of gene expression because there is no nothing to utilize it. Like if you have made lot of food at

home then there is a worth opening the you know dinner table or opening plates and putting water etcetera.

If no food is cooked, what will you do with dinner plate? There is no much of use by washing the dinner plate putting on dinner table it's a waste wasteful exercise right. Similarly, sigma 70 and RNA pol if they are coming together, they will turn on turn on a bunch of genes.

If this happened the medium should have enough nutrition to utilize it, but this 6s RNA if it is present, it will not allow the sigma 70 and RNA polymerase to act on genes promoter. They will come together, but 6s RNA will act like a bottleneck. It will act like a baffle for the gene expression even to continue. Rather it will turn on a different set of gene. It will not turn on the genes that typically a sigma 70 and RNA polymerase to will turn on rather the presence of 6 s RNA it will turn on a bunch of genes.

Just like in childhood you will be playing a bunch of games and once you grow like if you are 5-year-old or 10-year-old you will play a bunch of games. You will not play that game when you are 15 or 20-year-old and you will not play the same game when you are around 30-year-old.

So, once you cross 30 or 40 you will play some other game that is meant for your you know aging body do not need too much of physical activity those kinds of games and if you become really old you are 80 or 90-year-old still you can play game, but that will not be those games you can play maybe less muscular like playing some carrom board or chess or those kinds of games you can play.

But you cannot play a really you know tiring games once you are old. Same way once 6s RNA is there because stationary phase is the old phase or aged phase for the bacteria. So, it will turn on those genes that is going to be helpful and going to be quite useful for the bacteria to survive in the stationary phase. So, what we should understand here? The presence of this non coding RNA 6s RNA decides whether or not this bacteria is in log phase or stationary phase.

So, log phase 6s RNA is not needed if it comes, it should not come on to the sigma 70 and the RNA polymerase complex. So, 6s non coding RNA can modulate. So, this is what we should understand the modulation, but the same sigma 70 and RNA polymerase

2 you have here, but the presence of this 6s RNA modulate the target it is no more going to the target that is going to be helpful in the log phase.

Now, it is going to a different set of genes and promoters that is going to be helpful in the stationary phase. So, the take home message from here is that the presence of just one non coding RNA allowed the bacteria to get across this problem of log phase where you have plenty of nutrition and the lack of nutrition adequate nutrition in the stationary phase. But same sigma 70 same RNA polymerase 2 the difference was 6s RNA.

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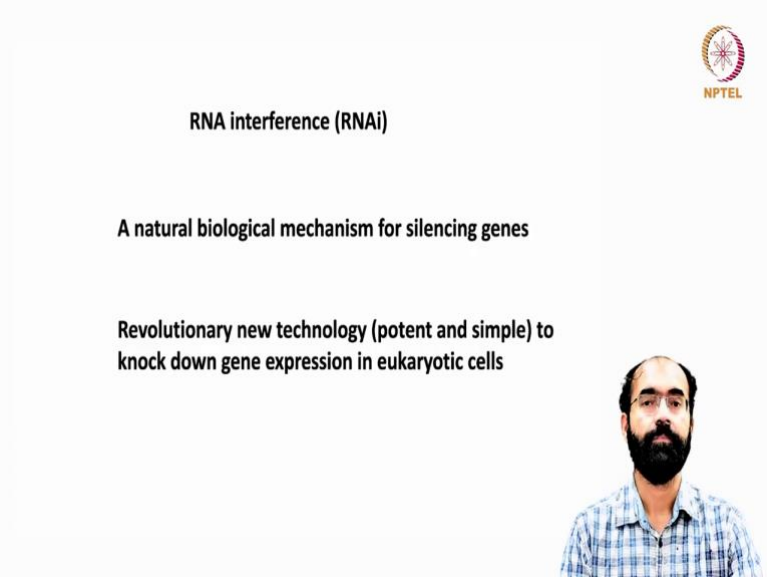

So, this is an example of modulation of protein function. Now, let us see more in detail about the RNAi. So, how RNAi has been discovered and how it has evolved. So, a natural biological mechanism for silencing the genes. So, RNAi is a way of living for organism it is not a novel feature it is a essential feature that is allowed for the organism to survive. Can be a relic from the RNA world itself or RNA world hypothesis that can be supported by this RNAi.

So, it is a revolutionary new technology it is very powerful, but it's extremely simple to knock down gene expression in eukaryotic cell. We saw some examples like we are talking about siRNA gene knockdowns etcetera siRNA they are all done with the help of RNA interference.

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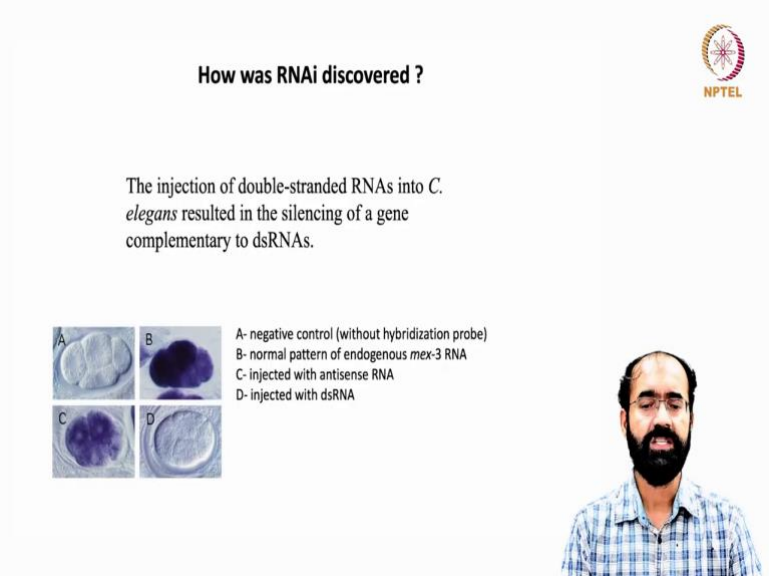

Let us see how is it discovered. How was RNA discovered? The injection of a double stranded RNA into C elegans resulted in silencing of a gene and this normally has to be complementary to a double stranded RNA. So, you inject a double stranded RNA of a given gene. Say gene A you inject a double stranded RNA against that gene then you will find gene silencing.

Let us see some examples. So, A is a negative control like when you do a in situ hybridization if gene expression is not there you will not see any color reaction because in RNA in situ hybridization is a very powerful technique to study the gene expression events. So, there is no color reaction because there is no gene expression whereas, in B its a normal pattern of endogenous that is mex -3 RNA. mex -3 is name of a gene and it is expressed quite strong.

In C same X 3 is there, but you injected with antisense RNA you have injected with mex -3 is a sense RNA and you inject it an antisense RNA whereas, in D what you did? You injected with double stranded MEX double stranded mex -3 RNA. Because of this it is mimicking somewhat like lack of expression like we saw in the case of A. So, that is a negative control. Negative control means no expression of mex -3.

So, it is now D is looking exactly like A. It is simply because you injected double stranded RNA that created very powerful and very strong RNA. Here also RNA has happened that is why it is weaker than B. B is very strong C is weak, but it is not nil

compared to A or D. D it is nil because not because there is no lack of there is it's not because there is lack of mex -3 expression.

mex -3 is there it is expressing, but it is not stable because it is completely degraded because you injected with double stranded RNA for mex - 3. So, we should understand that presence of double stranded RNA corresponding to a gene can create complete havoc in the stability of that RNA because that RNA will be marked for silencing.

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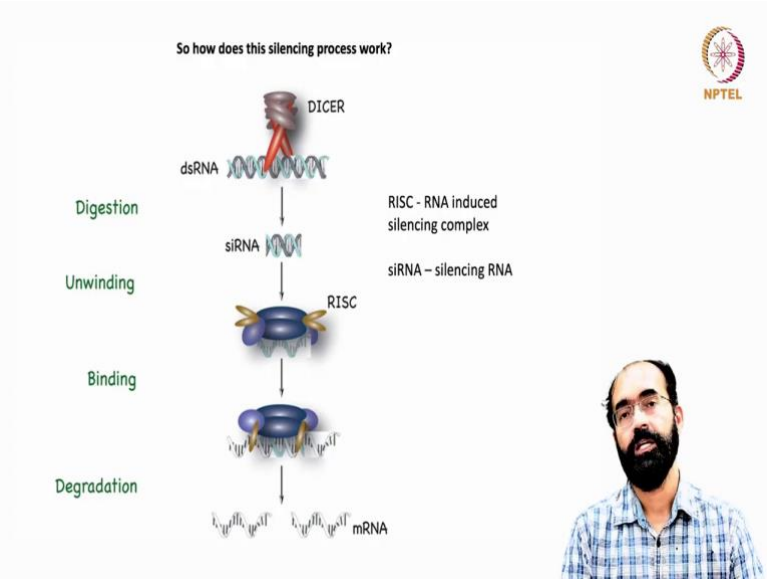

So, how does this silencing process work? So, kind of we have seen it the mechanism how the silencing works when we studied the micro-RNA because the DGCR8 and DROSHA trims the non-coding RNA to the pre micro RNA is created and it is in the nucleus and in the cytoplasm the Dicer which is a nucleus acts and gives rise to the actual micro RNA.

So, how a double stranded RNA enters the cell usually in the cytoplasm? Normally, RNA stays in the cytoplasm when you inject or deliver into any cell it stays in the cytoplasm because they do not go to the nucleus unless this RNA is forming an RNP that is meant for going into the nucleus.

So, the double stranded RNA when you put it into the cytoplasm, the Dicer acts on it and this is called digestion by the Dicer and this can trigger the formation of tiny RNA fragments and we call it as siRNA and this siRNA can cause the formation of RNA

induced silencing complex, we call it as RISC and This RISC can further give rise to degradation of the mRNA. So, you have digestion, unwinding, binding and degradation.

So, what happens with this micro-RNA being formed? The risk is formed because of RNA induced silencing complex and the siRNA acts as the nucleation point for the silencing event.

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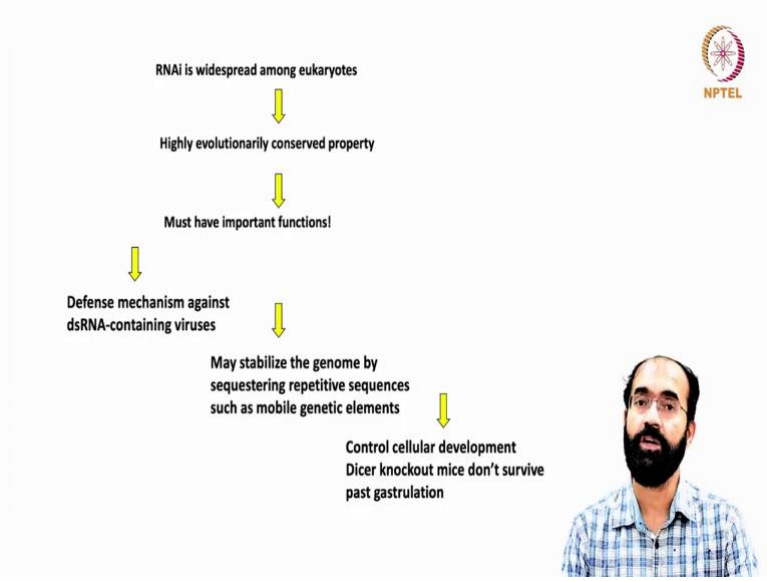

So, RNAi is a widespread mechanism among the eukaryotes. It is highly evolutionary and it is highly conserved. So, it is seen from C. elegans to humans and it must have important functions. If this mechanism is conserved across all these organisms, it must have some real relevance for the survival of this organism.

One mechanism is defense against pathogens. So, many pathogens such as viruses they have double stranded RNA. So, how will you distinguish your normal RNA versus a virus RNA? So, the best way of defending is I do not welcome any double stranded RNA. The cell says I do not want any double stranded RNA inside this cytoplasm. Whenever there is a double stranded RNA, it will chop off the entire RNA through the RNA induced silencing complex formation.

And it may stabilize the genome by sequestering repetitive sequences such as mobile genetic elements. This is another advantage; that means, it can help in stabilizing the genome by sequestering the repetitive sequence such as mobile genetic elements are

transposons. Many a times if transposons are present, they can cause damage to a normally functioning gene and can create you know trouble inside the inside the genes.

Like if a transposable element entered into a normal gene, then it will get disrupted. So, many a times when you have this tiny RNA, siRNA present they will find the mobile genetic element attractive go and pair with them and cause their silencing.

And the third function is the controlled cellular development and normal like during development like I told you if a given gene has to be turned off immediately. Of course, transcription can be stopped, but the RNA is already there in the cell. So, you need to get rid of this you need to get rid of this RNA quickly.

So, this is possible if you have an RNAi acting onto that mRNA. So, that is part of normal development. So, Dicer knockout mice do not survive post gastrulation. That itself shows how important the Dicer because absence of Dicer prevents the formation of tiny siRNAs and microRNAs.

So, Dicer is a must to be present for this microRNA to form. So, if you knockout the Dicer you do not get the animal surviving beyond gastrulation. Means very early search of development itself Dicer is essential. So, we will study more in detail about this RNA induced a silencing and more important roles played by the non coding RNAs in the next class.

Thank you.