RNA Biology
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Lecture - 47
Dosage Compensation, Xist and ncRNA in Imprinting: Mechanism of RNAi in Action

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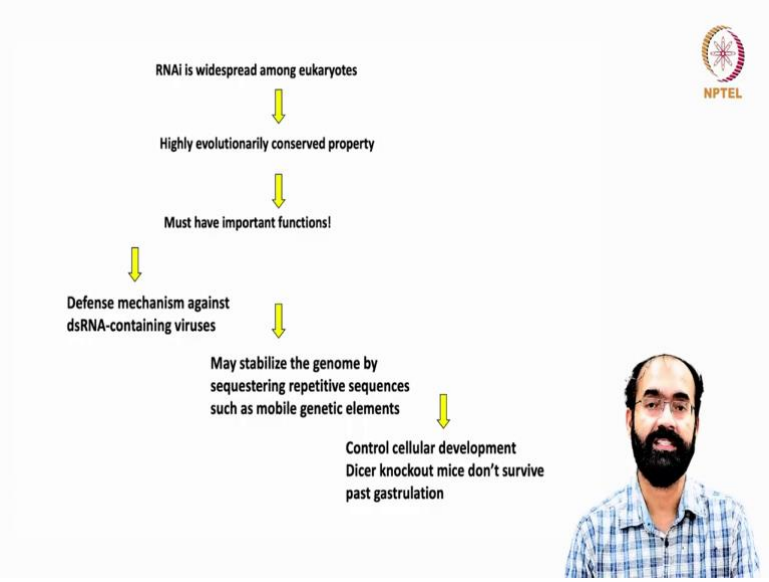

Hello everyone, welcome back to another session of RNA Biology. So, we were here in this last slide and we mentioned three major functions undertaken by RNAi that is defence mechanism against double stranded RNA containing viruses and majority of the viruses have RNA as their genome and double stranded RNA as their genome. So, that can be a very helpful defence.

And it can stabilize the genome by sequestering the repetitive sequences such as mobile genetic elements that can create havoc in the organisms survival. So, third function is the normal development and the dicer knockout do not cross the gastrulation stage that itself indicates how important the RNAi mediated gene regulation events right from the early embryonic development.

And in one of the classes, we also discussed the importance of let 7 and the Lin 28, Lin 28 can completely get rid of the let 7 micro RNA that is another way of regulation; that means, some events some gene expression events has to happen you have to handle this

siRNA completely you have to get rid of this siRNA so, that the required genes can be expressed.

So, that is the other side of the spectrum what you are talking about the other side of the table. Normally, many protective functions are there sometimes siRNA will do more than what it should that avoidance of an siRNA becomes important for the expression of a given gene.

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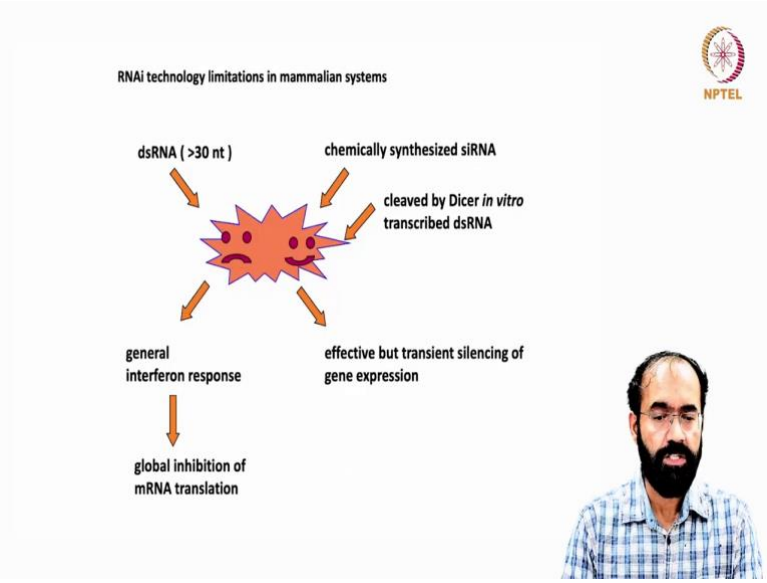

So, RNAi can be used as a tool in various research also. So, RNA technology and limitations in mammalian systems we can address how does it happen how is it is carried about. So, many a times you can synthesize an siRNA chemically synthesize in a laboratory and deliver into a cell line or an organism or a tissue what you are interested in etcetera.

And this double stranded RNA must be around more than 30 nucleotides long. So, it can follow two major directions; one is if the double stranded RNA is longer than 30 then it can create a general interferon response. Interferon is chemicals induced after a viral infection or viral injury. So, body induce interferons to counter the action of viruses and this can trigger eventually trigger global inhibition of mRNA translation.

So, no cell will produce any RNA. So, complete block ad including the virus. So, it is like you may have seen in if you are following the economy like if there is inflation,

there is too much of money. What is inflation there is too much of money in the market and when too much of money is there in the market the price for goods will go up and they are interdependent.

So, what is the possible way of controlling it? One way is to control that you know you print larger currencies one way other option what many a times the Central Banks like in India it is the Reserve Bank of India. What they will do? They will increase the interest rate like you may have heard about this repo reverse repo like that.

So, because of this what happens the governments will get more money deposited in the Reserve Bank because the interest rates are going up so, naturally the money from the market, from the people from your wallet it will automatically flow into the banks because more interest rate is there. So, you are compelled to withdraw the money from the market into the banks.

So, that automatically since you have less money in your pocket your demand also will come down. So, automatically the price for the goods will come down. Why I said this because, this is a strategy once interferons are turned on the overall translation is blocked. It may sound counterintuitive, but in a short span of time this helps. So, just like you withdraw the money from the market so that you do not spend much because you do not have money, how will you spend it?

So, naturally the demand for goods will come down and the price of the goods will come down. So, you control the inflation. So, this there are different strategies are there. So, one of them what I was mentioning that is what governments will do. So, the interferon response is triggered if the more than 30 nucleotide double stranded RNA is present in the system in a cell then there is a global inhibition of mRNA translation takes place.

And if you put chemically synthesis siRNA or it can be even cleaved by Dicer in vitro for transcribed double stranded RNA. If you put a double stranded RNA inject like you saw in the previous class an example of demonstrating the anti-sense RNA by injecting the double stranded RNA into the cells.

So, double stranded RNA if you are putting in you have to induce the Dicer you have to get the action of the Dicer to cleave the RNA into tiny fragments which will completely annihilate the mRNA corresponding to that double stranded RNA it will not stop all the

RNAs present in the cell, but it is very specific. So, you can either put siRNA to block the expression of an mRNA or you inject a double stranded RNA both will bring down the gene expression that is the protein translation corresponding to that mRNA.

So, effective, but it is very transient silencing of gene expression siRNA or injection of double stranded RNAs only good as long as they are available in the cell there is no constant production is happening it is just like I give you food for today that will not settle your life for life long right for today that is ok until that food is digested you will be healthy and ok but tomorrow it's a question.

So, siRNA and double stranded RNA way of regulating the gene expression is transient, but many cell culture studies that is good enough because you are culturing the cells in a you transfer them with siRNA good dose of siRNA. So, as long as your experiment is carried out you will have the knockdown phenotype of that particular gene and you can study the importance of that gene or protein etcetera.

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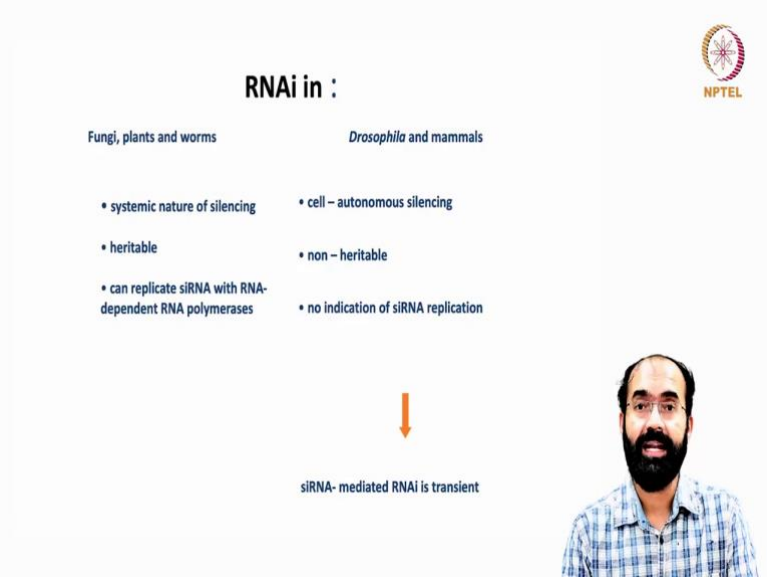

So, RNA can be further elaborated in other model such as fungi, plants and worms and it is also well characterized in drosophila and mammals. So, in fungi, plants and worms they are systemic nature of silencing and it is heritable; that means, go from one organism to another and it can replicate siRNA with RNA dependent RNA polymerase.

So, these are normally seen in fungi, worms and plants. What are they? They are systemic in nature; that means, it is the rule is concrete that is systemic means it is unchallenged it will be following the knockdown very effectively. And heritable means it can go from one organism to another; that means, you think heritable means like if your father or ancestors have a big house you inherit it and then maybe your son or grandson will inherit it.

So, this is called heritable. So, this go from one generation or one cell to next cell or one generation to another. So, that is called heritable nature and it can replicate the si RNA with a RNA dependent RNA polymerase in these organisms. Whereas, in drosophila and mammals it is cell autonomous silencing; that means, it is dependent on which cell type or which cell you are talking about.

Autonomy means what? Like you can say every house in India we can say that they are autonomous means house A and house B house A may be non-vegetarians house B may be vegetarians there is no rule that says that ok everyone has to follow a particular rule you know you should be eating breakfast should be this, lunch should be this, dinner should be this no.

You can choose what you want to eat. So, this is called autonomy. Same way the RNA interferon's in drosophila and mammals it is autonomous; that means, a given cell based on which cell you are talking or which is cell you are discussing it can have a phenotype. It cannot happen that you drop the ball from your hand it will go and hit the ground that is called systemic nature, ball will not fly if it is a balloon, you release it may fly based on what gas is filled in.

So, it varies right same way the nature of the gene silencing varies from cell to cell in case of drosophila and mammals. And it is non-heritable means, every cell has to produce its own siRNA machinery, it is not heritable. Cell A when it give rise to two daughter cell it cannot inherit if some small quantity if it is present may pass on, but that we have a dilution effect.

So, unless the daughter cells also produce on their own they will not have a heritable feature. No indication of siRNA replication means, siRNA cannot make a copy from an existing template, we cannot make a copy of an siRNA in drosophila and mammals from a pre-existing siRNA.

The main reason is none of this eukaryotes such as drosophila and mammals produce RNA dependent RNA polymerase they are normally produced from viruses. And siRNA mediated RNAi is transient; that means, it is not a permanent feature normally in all this organism.

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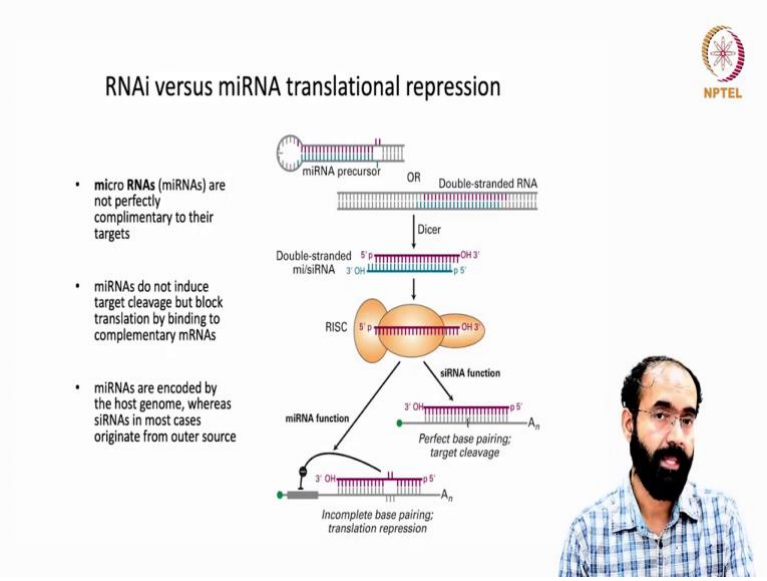

So, now let us understand RNAi versus miRNA translational repression. miRNA we have seen it drosha and dicer they cause the production of miRNA the main difference is the way of pairing the target. siRNA and miRNA work more or less the same, outcome is same, but the difference is in the nature of pairing nature of target selection. So, let us see how does it work. So, this stem loop structure is a miRNA precursor.

And this can stay either in this form like a stem loop structure or it can form like a two linear RNA or in a double stranded RNA. So, this stem loop structure has a double stranded region and a loop whereas, here it is two long strands that is paired together. in either case both this or this both can be acted upon by dicer and you end up getting double stranded micro RNA and they are shorter in length, they are of the order of 22 nucleotides and they have a 3 prime OH and a 5 prime phosphate on both the ends.

This double stranded micro RNA and we can call it as an siRNA also because there is no much difference between the two except for the target binding, except for the mechanism in which they go and recognize a target. So, they need to form RNA induced silencing

complex. It is a group of the RNA induced silencing complex is formed by a group of proteins and one of the major protein of that complex is argonaute.

Argonaute protein has to be present in a cell abundantly without which this risk complex formation and silencing can be compromised. So, siRNA when it has to work in this RISC it has to be a perfect binding look here you can see they are paired base by base the entire 22 nucleotides have paired base by base whereas, if it has an miRNA mediated function you need not have to have a perfect that is called incomplete base pairing and or improper base pairing.

Improper here what I mean is the base pairing is happening as it should be, but it is not continuous it is not complete. So, in siRNA it is pairing completely and it will eventually cause the target cleavage. What is the target? mRNA is the target. So, siRNA can go and bind on to specific regions in mRNA and cause the target cleavage that is the main function of the siRNA.

Whereas, micro RNA what they do? It cause the incomplete base pairing and it will cause the translation repression. So, it will prevent the translatability because it is bound down to an mRNA and it is an incomplete binding, but this incomplete binding can cause a translation repression. So, this mRNA is not degraded, but it is not allowed to produce the protein.

But outcome is same whether you it is almost like you know you did not allow a person to speak you can either do not give him the right to speak or you give him the right to speak, but do not give him a microphone or you put some cloth in his mouth. So, outcome is same ok you can speak, but I will not allow you to allow voice to come out. So, in one case siRNA you are degrading the mRNA itself no question of translation in the case of micro RNA.

But pairing is different pairing is not complete as in the case of siRNA incomplete pairing because of this incomplete pairing it will prevent the translatability of this mRNA that is how they work. So, micro RNAs are not perfectly complementary to their targets; targets means mRNA.

And micro RNAs do not induce target cleavage, but block translation by binding to complementary mRNAs and micro RNAs are encoded by the host genome whereas,

siRNA in most cases originate from outer source; that means, you have to supply or it has to come from some you know viruses or some other sources. Normally micro RNAs are produced endogenously siRNAs either artificially made or it is put into the cell from an outside entity.

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So, you can read this article that is published in nature that is about TRBP that is recruiting the Dicer complex to Ago2, Ago means Argonaut for micro RNA processing and gene silencing it is a very interesting paper. So, if you read you will find it more exciting about this RNA induced silencing or RNA interference.

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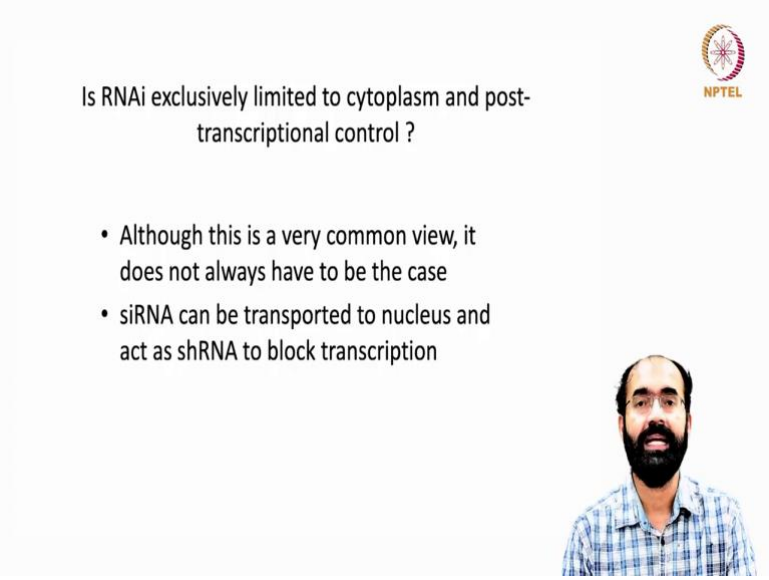

Is RNAi exclusively limited to cytoplasm and post-transcriptional control? That is a big question you can. Although this is the common view, we have it does not always have to be the case; that means, RNAi although functions in the cytoplasm it can have other long lasting effects also. Let us see some examples. siRNA can be transported to the nucleus and act as shRNA to block the transcription itself means transcriptional gene silencing.

Many cases has been reported that RNA the shRNA can prevent the transcription in the nucleus itself. So, this is something interesting that how siRNA or micro RNA or the RNAi the phenomenon of RNAi effects the transcriptability in the nucleus and RNA degradation or translation inhibition in the cytoplasm which is quite interesting.

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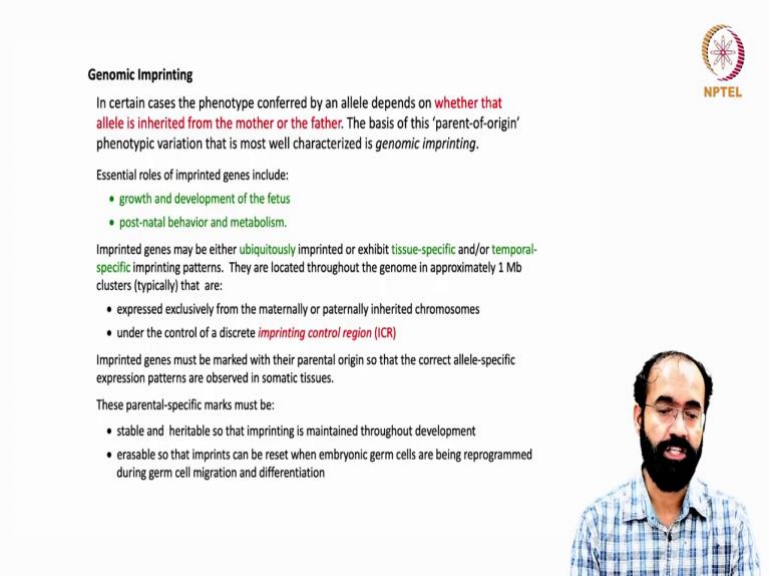

Let us come back to the genomic imprinting because this non coding RNA have got lot of role in the genomic imprinting. In certain cases, the phenotype conferred by an allele depends on whether that allele is inherited from the mother or father this we have discussed sufficiently that imprinting itself means either paternal copy or maternal copy should be allow to express not other way around.

So, the basis of this parent of origin phenotype variation that is most well characterized is called genomic imprinting. Genomic imprinting itself means that the parental origin has got a big importance and big value in deciding how a gene is allowed or whether a gene is allowed to express.

Essential roles of imprinted genes what are those so called imprinted genes and what they do? So, that is a question we should have. The growth and development of the foetus; foetus means the early embryo that is what we call it as foetus that the growth and development of the foetus is regulated by imprinted genes and postnatal behavior and metabolism. These are all the two major category of functions that is governed by the so called genomic imprinted genes.

So, imprinted genes may be either ubiquitously imprinted or exhibit tissue specific or temporal specific; that means, time specific. Normally you may have heard about spatial and temporal. Spatial means space, tissue specific means spatial means your heart is somewhere, liver is somewhere, kidney is somewhere, brain is somewhere.

So, the space is deciding temporal means time, age whether are you in the embryonic stage are you 10 year old, are you 20 year old like; that means, you means your organ your if you are 10 years old your liver is 10 years, old your kidney is 10 years old something like that. So, the time dependent manner and embryonic development also a given gene is required for the embryonic development.

So, a given gene is not doing the same function on the first day of development, second day of development or third day of development. So, that is called time dependency temporal manner. So, they can work both in a spatial and temporal fashion and they are located throughout the genome in approximately 1 Mb cluster means, 1 mega base or 1 million base clusters typically that are expressed exclusively from maternally or paternally inherited chromosomes.

So, the imprinted genes are not an isolated island although they are secluded from the rest of the chromosome, but the area is pretty big you know 1000 base is 1 kilo base 1000 kilo base is 1 mega base. So, that much widespread span the effect will be there and that are these imprinted genes of this 1 Mb clusters are under the control of discrete imprinting controlled region we call it as ICR Imprinting Controlled Regions govern the ownership of or the control the overall expression of this entire region.

So, imprinting controlled region is somewhat similar to that of the regulatory elements we studied in case of eukaryotic and prokaryotic promoters, but imprinting controlled region decides whether the this area or this bunch of genes in this area should be allowed to express or not. So, imprinted genes must be marked with their paternal or parental origin not paternal parental origin.

So, that the correct allele specific expression patterns are observed in all the cells originated from that particular zygote or the initial cell onwards. So, the parental origin has to be followed right from the beginning until all the different tissues are formed from this single cell to start with. So, these parental specific marks must include and they must be stable and heritable.

So, that the imprinting is maintained throughout the development. Just like you saw the ex chromosome inactivation it is a good example of imprinting once a ex chromosome is inactive it has to be maintained so in all the cells originating from that particular cell. So, cell A gave rise to two daughter cells and those daughter cells gave rise to further

daughter cell they should follow the same pattern if it is an imprinted gene so, it should be stable and heritable.

But it also should have another feature erasable so that imprints can be reset when embryonic germ cells are being reprogrammed during germ cell migration and differentiation. So, this is something important if imprinted genes remain imprinted throughout the lifespan even during the germ cell formation like in the case of female it is eggs in the case of males it is sperm during that time also this imprinted genes are remaining imprinted then many genes can be lost and the organism suffers.

So, there is a exception to this rule. What is that rule? This imprinting has to be reversed or it is revisited or they are erased in the formation of the germ cells. If this does not happen then you lose that particular because first of all it can be problematic the germ cells you are contributing only one copy.

And you are contributing a gene that is already imprinted then that organism can suffer what if you are contributing an imprinted gene that is supposed to be expressed. In the next generation then that will be problematic. So, what usually happens is during the germ cell formation the imprinting is reversed there is no imprinting maintained in germ cells.

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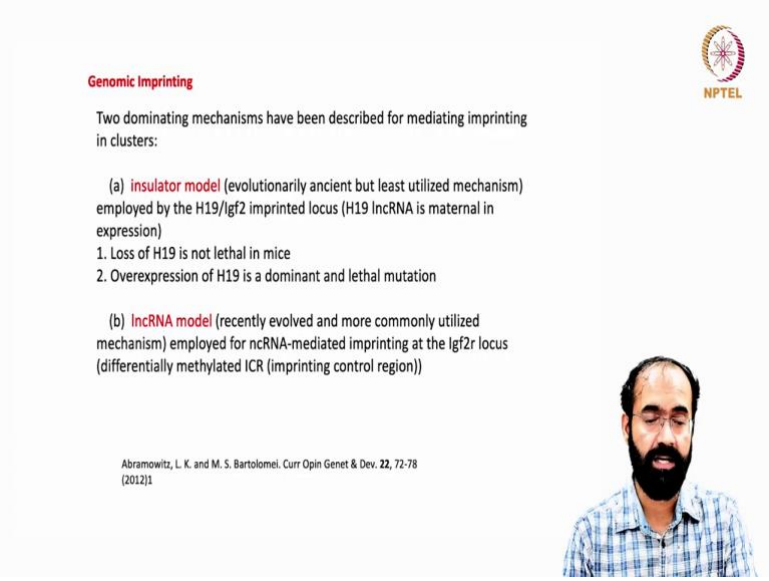

So, genomic imprinting there are two dominating mechanisms have been described for mediating the imprinting clusters. Like we already said the imprinting controlled region is playing a major role in governing the status of imprinting. So, one model is insulator model that is it is an evolutionary ancient, but least utilized mechanism and it is employed mainly by one famous pair of gene that is H19 Igf2 and Igf2 is the gene H19 is a non-coding RNA.

And this imprinted locus is studied a lot and a lot of people have used this as a tool for understanding the importance of this imprinted locus. So, H19 lnc long non-coding RNA is maternal in expression. So, it is originated from the maternal copy all the paternal has father also have this gene, but it is never expressed. So, the loss of H19 is not lethal in mice, but the overexpression of H19 is a dominant and lethal mutation.

So, what do we understand H19 absence may not cause that much of a problem, but overexpression can lead to loss of gene function some gene function is affected and that gene is Igf2 and another model is lncRNA model that is model number 2 that is it is recently evolved and more commonly utilized mechanism recently means evolutionary timescale recently not that you know couple of years or something it is for past few thousands of years it is the latest evolved mechanism that is lncRNA model.

So, it is employed for non-coding RNA mediated imprinting at the Igf2r, r stands for receptor Igf2r locus that is differentially methylated ICR imprinting controlled region. So, what we should understand the genomic imprinting can follow either an insular model insulator model that is an old model and a lncRNA model which is a new model.

So, we will revisit the genomic imprinting more in detail in the subsequent classes and we will have a better understanding of this topic and because genomic imprinting is a one of the most stringently controlled and one of the most important way of gene regulation in all higher eukaryotes such as mammals. I will stop the class now and we will continue on this topic in the next class.

Thank you.