RNA Biology
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Lecture - 45
Dosage Compensation, Xist and ncRNA in Imprinting: The Roles of YY1

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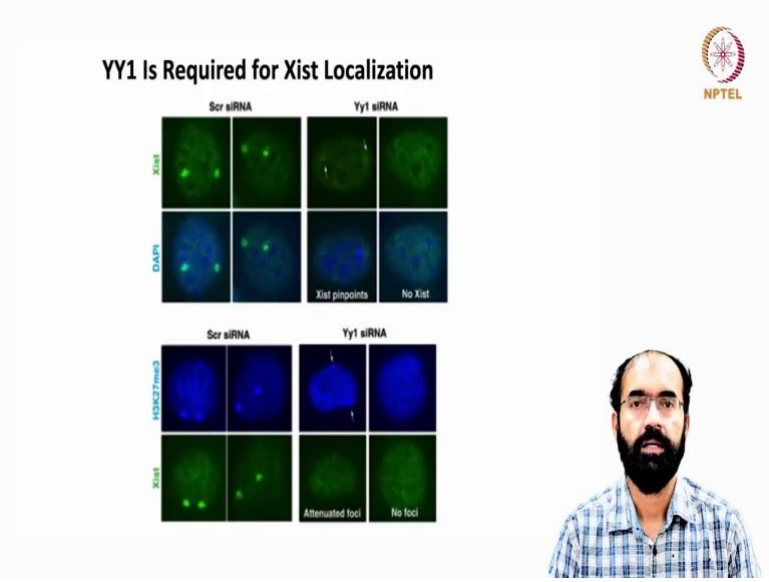

Hello everyone. Welcome back to another session of RNA Biology. So, we were here in the previous class that we were discussing about the role played by a protein YY1 that is also called as Yin Yang I. This protein helps in tethering the Xist onto the chromosome, the place where it is being produced. So, if you use YY1 siRNA to block its translation then you will not have the ability of this Xist localized onto the corresponding chromosome.

So, that is and also the methylation status H3K27 tri-methylation, which is a post tethering or post Xist attachment onto the X chromosome, then only you get this H3K27 tri-methylation markers, so that will go missing. So, the take-home message from these experiments is that Xist will be able to attach to its targets that is the X inactivation center with the help of a protein called YY1.

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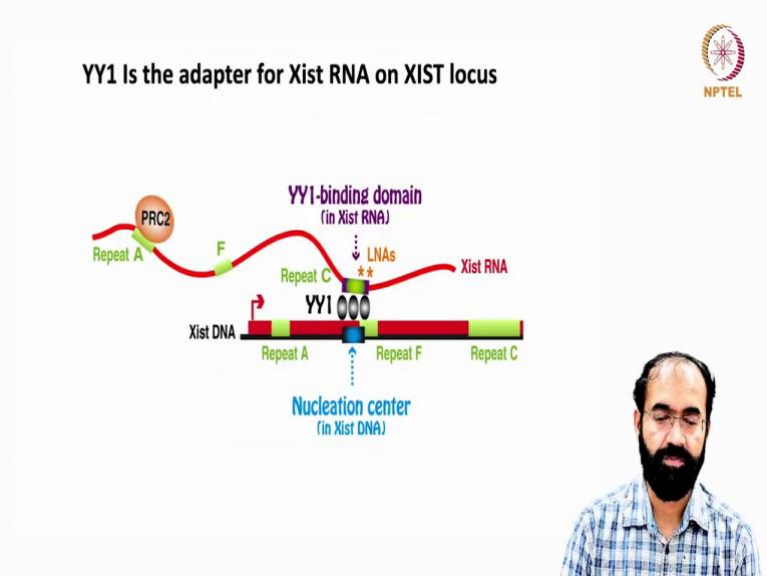

So, how is the mechanism of tethering or attaching? So, it works something like this. So, you have this Xist RNA and this is the place where it is supposed to be pairing the nucleation center the X inactivation center and the protein YY1 is right in here in the middle. So, somewhat like a glue like a Fevikwik or Fevicol glue that is attaching onto it. And once it is attached then that is triggering the further events such as PRC2 recruitment for the S3K27 tri-methylation etcetera.

So, the YY1 binding occurs onto the Xist RNA through a specific domain called YY1 binding domain that is present on the Xist RNA. So, Xist RNA will be able to bind with the YY1 protein and YY1 protein in turn have an affinity for binding onto the X inactivation center and you can see the PRC2 complex also have a location on the Xist RNA. So, this brings in the PRC2 complex.

So, to recap this whole thing this is the X chromosome, this is the X inactivation center and from the X inactivation center you have the production of Xist RNA. And Xist RNA have got YY1 protein binding site and YY1 protein is able to bring the Xist RNA onto the X inactivation center that is the nucleation center and this Xist in turn has got binding sites for PRC2 complex, polycomb repressor complex 2 which is required for the S3K27 tri-methylation. So, this is the whole process.

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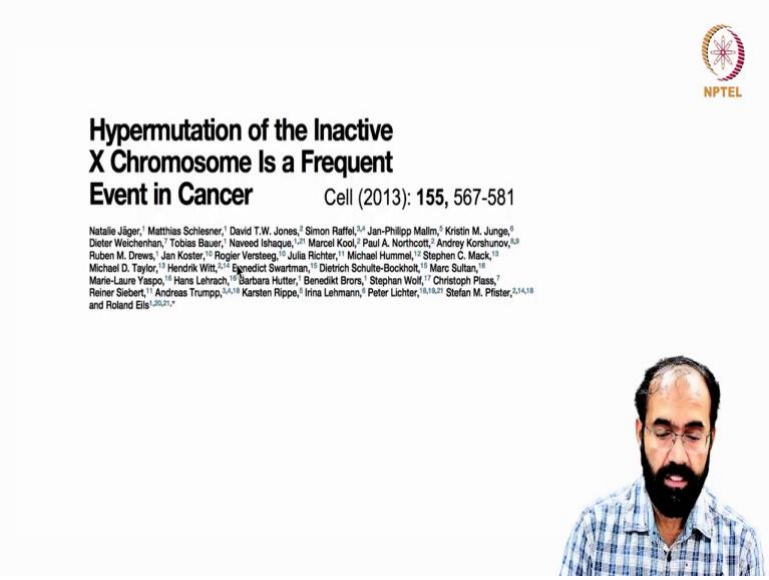

Those who are interested to know more about the importance pertaining to the X inactivation center, can read this article that is published in cell few years ago. And this paper discussed one more important thing that is the mutation rate is seen to be very very high in the inactive X chromosome like this, so called Barr body which is meant to be not functional is accumulating.

Lots of mutations compared to the counterpart that is the X active or active X chromosome have got less mutations or it is just like any other normal chromosome. Whereas this inactive X chromosome accumulates lots of mutations and we call it as hyper mutations. Let us see with some more detailed observations.

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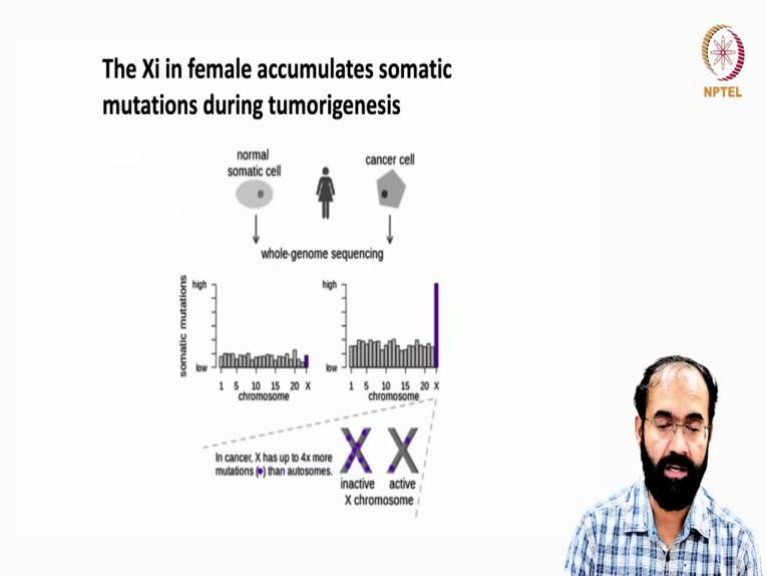

So, the X inactive means non-functional X chromosome in females accumulates somatic mutations during much more during the tumorigenesis. Let us see a normal cell and a cancer cell ok. So, in a female if you have cancer those cells also have got 2 X chromosome one is active one is inactive.

So, we are comparing the inactive and active and how does it differ. So, in this graph you have somatic mutation. What is somatic mutations? Mmutations that is occurring in a housekeeping gene or a gene that is responsible for the normal day to day functioning of the organism. So, that is what we refer to them as somatic mutations and the other genes are meant for determining the sex of the organism.

So, they are not called somatic genes, they are sex specific genes. So, the somatic genes can be present in the X chromosomes also. And the somatic mutations are often leading to some serious trouble in the organism's survival and other day to day affairs. So, what you have to see here is, the number of mutations bottom most of this Y axis is low, top most of this Y axis is high; that means, low number of mutations means the graph will be restricting towards the bottom. Whereas, large number of mutations means it will be pretty high.

So, the somatic mutations across the chromosome starting from chromosome 1 until 20, 21, 22 and this is the 23rd in human that is the X chromosome. So, you can see here in X chromosome the there is more of the mutation; that means, there is the X chromosome is

shown in this blue color. So, what happens? The same female cell, same means not the same individual another female cell that has got mutation rate in somatic chromosome or somatic genes and compared to the X chromosome.

So, you can see here, compared to a normal healthy cell, the number of mutations are pretty low and you can see throughout the chromosome, it is almost double. You have some way or the other mutations because if it is not a normal cell it is a cancer cell. But what you have to see is, when all the somatic chromosome or somatic genes have got almost double; whereas, the X chromosome it is almost 4 times more mutations.

So, when somatic chromosome have got two times mutation compared to a healthy normal cell, the cancer cell have got almost double compared to a normal cell, but the same cancer cell the inactive X chromosome usually will accumulate a plenty of the mutations almost 4 times more. So, in cancer X has up to 4 times more mutations than in autosomes. And one more thing if you see in X chromosome which is inactive and active if you compare, the inactive one has accumulated more number of mutations compared to the active one.

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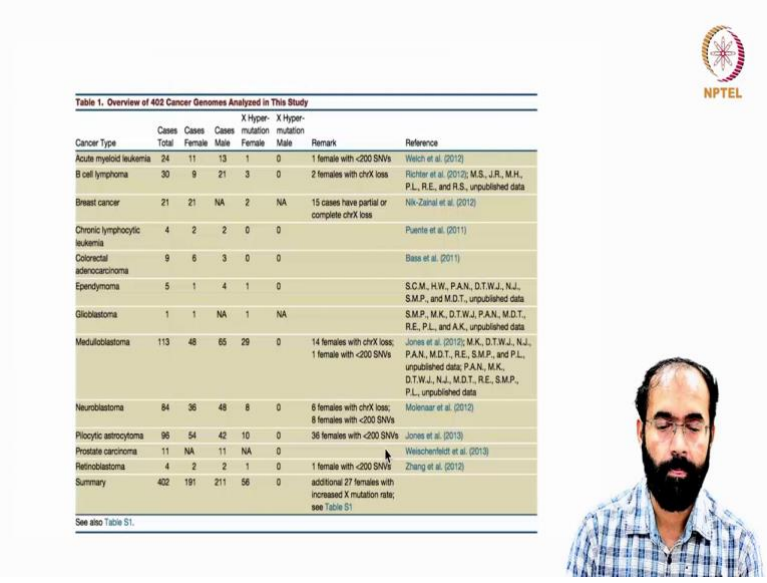

Table 1. Overview of 402 Cancer Genomes Analyzed in This Study

Cancer Type	Cases Total	Cases Female	Cases Male	X Hyper-mutation Female	X Hyper-mutation Male	Remark	Reference
Acute myeloid leukemia	24	11	13	1	0	1 female with <200 SNVs	Welch et al. (2012)
B cell lymphoma	30	9	21	3	0	2 females with chrX loss	Richter et al. (2012); M.S., J.R., M.H., P.L., R.E., and R.S., unpublished data
Breast cancer	21	21	NA	2	NA	15 cases have partial or complete chrX loss	Nik-Zainal et al. (2012)
Chronic lymphocytic leukemia	4	2	2	0	0		Puente et al. (2011)
Colorectal adenocarcinoma	9	6	3	0	0		Bass et al. (2011)
Ependymoma	5	1	4	1	0		S.C.M., H.W., P.A.N., D.T.W.J., N.J., S.M.P., and M.D.T., unpublished data
Glioblastoma	1	1	NA	1	NA		S.M.P., M.K., D.T.W.J, P.A.N., M.D.T., R.E., P.L., and A.K., unpublished data
Medulloblastoma	113	48	65	29	0	14 females with chrX loss; 1 female with <200 SNVs	Jones et al. (2012); M.K., D.T.W.J., N.J., P.A.N., M.D.T., R.E., S.M.P., and P.L., unpublished data; P.A.N., M.K., D.T.W.J., N.J., M.D.T., R.E., S.M.P., P.L., unpublished data
Neuroblastoma	84	36	48	8	0	6 females with chrX loss; 8 females with <200 SNVs	Molenaar et al. (2012)
Pilocytic astrocytoma	96	54	42	10	0	36 females with <200 SNVs	Jones et al. (2013)
Prostate carcinoma	11	NA	11	NA	0		Weischenfeldt et al. (2013)
Retinoblastoma	4	2	2	1	0	1 female with <200 SNVs	Zhang et al. (2012)
Summary	402	191	211	56	0	additional 27 females with increased X mutation rate; see Table S1	

See also Table S1.

So, this is proven fact in across a bunch of cancers. So, it is an overview of 402 cancer genomes analyzed for this study in that paper, that cell paper. So, you can see I will not go through each and every cancer that is acute myeloid leukaemia, B cell lymphoma, breast cancer so and so.

So, you can see, the number of female study and number of mutations studied or analyzed and several other related publications are also cited here. So, what we understand that in human female the mutation rate is much much high in the inactive X chromosome of a cancer cell or cancerous phenotype. So, that is what this study has proven.

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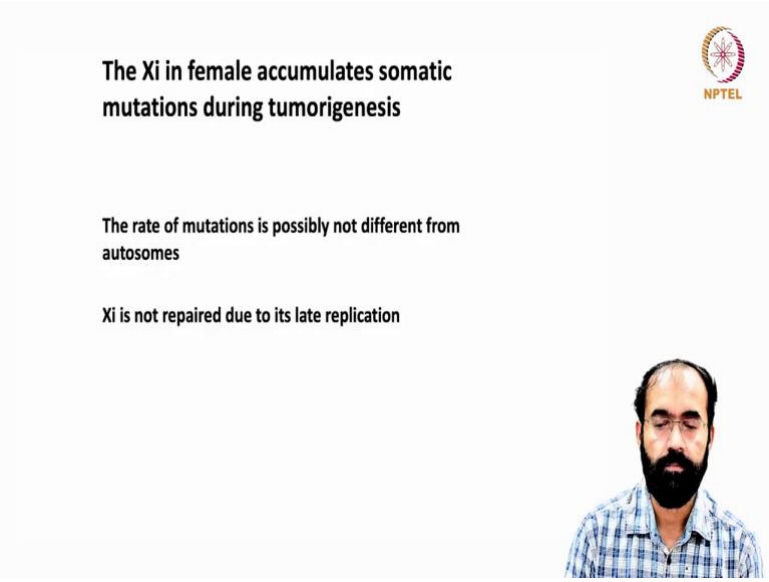

So, the inactive X chromosome in female, accumulates somatic mutations during the tumorigenesis. We kind of know the reason, because we know because of the high level of compaction of this chromosome it is taking lot of time to unpack it and because of this towards the end of S phase. S phase is the synthetic phase, that is the phase where the DNA replication takes place, that is the phase where there is any error in the DNA replication that has to be monitored everything happens in that phase.

However, if this fails, if it could not do every chromosome is vulnerable for changes. The so-called proofreading activity helps a lot in fixing the errors. But what happens, since the inactive X chromosome takes lot of time to unpack it, it do not get enough time for repairing the possible damages. So, if there is a damage that will be maintained as it is, there is no time to repair it, it will move on to the G2 phase and then M phase.

But it is not a deleterious thing in inactivate X chromosome because none of these genes are expressed. Hence, there is no one to take care there is no mechanism to take care of fixing the mutations in the inactive X chromosome. So, the rare mutations is possibly not

different from autosomes the X inactive or inactive X chromosome is not repaired due to its late replication.

Because in order to repair you need to have enzymes available in the cell, because since it is almost towards the end of the S phase, the enzymes required for repair are missing. But soon after the replication is done, the X chromosome is made inactive immediately. So, there is no scope for repair and hence it prevail the mutations as it is.

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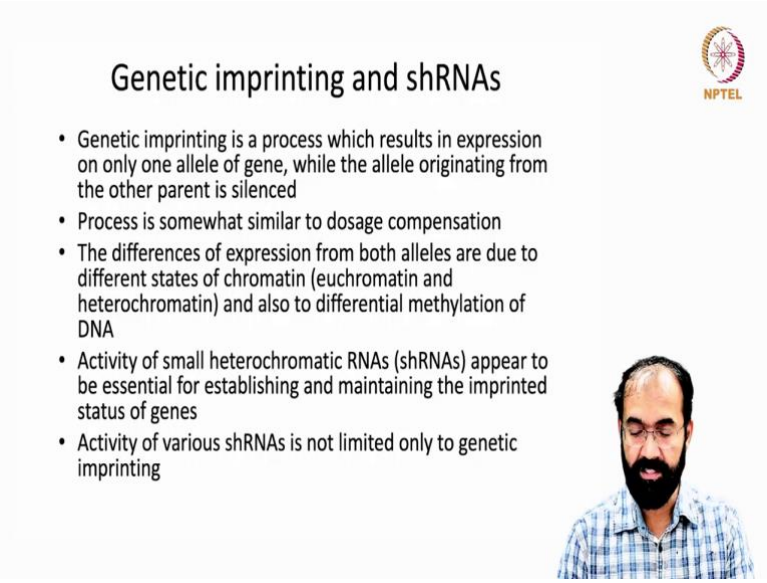

So, now let us see, how the genetic imprinting is undertaken by shRNA. So, we know to some extent that what is genetic imprinting. I gave some examples in the previous class also. Imprinting basically means; you are designed, you are guided, in a particular manner; that means, you have to do so and so. Like childhood from childhood, you have been given some specific mannerisms or manners you have been taught.

And you follow accordingly say in school you say you have to wish good morning to your teacher and like that teacher wherever you see in any time later in your life you will still learn to wish. So, it is almost like a imprinted behavior or imprinted character, and no matter what you will stick with it. So, many times many genes also follow an imprinted behavior, when it comes in terms of expression. So, this non coding RNA is contribute to a large extent in controlling the imprinted characteristics.

So, genetic imprinting is a process, which results in the expression of only one allele of a gene and that is called mono monoallelic gene expression. While the allele originating from the parent, other parent the other parent is silenced. We have to understand you have two copies of each gene and we call it as allele, say gene A you have two copies of it one came from father, one came from mother.

So, we call them as a paternal copy and maternal copy these are alleles. For many genes the copy that came from father will be allowed to express. And for many genes the copy that came from mother will be allowed to express. In some cases both the copies are expressed and we call them as bi-allelic gene expression, when only one is allowed to express that is called monoallelic gene expression.

But this monoallelic gene expression to a large extent is imprinted. Imprinted means it can not happen that for a given gene ok, like X chromosome we saw that randomly one of the X chromosomes inactive much later stage in the embryonic development. Earlier it is the paternal copy that is made inactive X chromosome.

But when it comes to imprinting it cannot be random. Some genes, many examples we will see them some genes a given gene it will be expressed, if it is came from if it is coming from the father; whereas, for some other gene it will be expressed, if it is coming from mother.

So, what can be outcome? Let us think this way, say paternal copy had got some issue. It is not correct it is having some mutations; the maternal copy is perfectly fine. So, you have an option of expressing the good copy, but it will not happen because this gene is following imprinting rule.

So, if the paternal copy is supposed to be expressing for that particular gene, if that paternal copy is having some defect then you suffer you have problem, although you have a perfectly ok maternal copy with you, that copy is not allowed to express. So, this is the importance and beauty of the imprinting. So, many diseases occur in the in a person because of this imprinting.

imprinting do not cause the disease because of the condition like I told you, because that gene is supposed to express only from father or so and so gene is expressed only from mother. If the father or mother gave you a mutated copy, then there will be a problem in

in terms of it's you know functionality or based on how severe the phenotype is the organism suffers.

So, imprinting is not just a way of living sometimes it can cause really deleterious effect. The process is somewhat similar to dosage compensation, imprinting we studied imprinting to some extent while discussing the dosage compensation also. The differences of expression from both the alleles are due to different states of the chromatin.

chromatin we know can be of two types; euchromatin and heterochromatin. Euchromatin means lose chromatin and DNA is accessible for transcription. Whereas, heterochromatin means it is tightly packed chromatin, where the gene expression is poor or nil. So, the chromatin is not opening up, DNA is not accessible. So, that is called heterochromatin.

So, the differences in expression from both the alleles are due to different states of the chromatin and is also having differential methylation of the DNA itself. We have seen H3K27, histone 3, lysine 27, tri-methylation in the case of X inactivation and many a times it is associated with repressive histone modification that is H3K27 methylation; wwhereas, H3K27 acetylation is an indicator of gene expression active gene expression.

So, we should understand that the differences in the gene expression events to a large extent goes to the imprinted status of a gene and not all genes are imprinted. A fraction of the total genes are imprinted. Activity of small heterochromatic RNA, we call them as shRNAs appear to be important for establishing and maintaining the imprinted status of the gene.

So, like X chromosome we have seen it making an X chromosome inactive is the first part only, but you have to keep maintaining, if you fail to maintain it then that can lead to trouble because dosage compensation will be imbalanced. Some situations similar to that of down syndrome can come, not exactly the same thing like down syndrome is happening mainly because of lack of such dosage compensation, you have 3 copies of chromosome number 21.

So, something similar can happen if there is a leaky expression of X chromosome. So, same problem can happen if there is a leaky expression of one of the imprinted gene say,

a paternal copy has to be turned off you do not want that gene to be turned on, if you turn it on due to whatsoever problems, then that can lead to an imbalance in the dosage compensation.

So, what we should understand, genomic imprinting is a way of ensuring dosage compensation and it is also a way of ensuring heterozygosity; that means, heterozygosity here I am referring to as the outbreeding. Like many species, like you may have heard about, like some reptiles, like you may have heard about this Komodo dragon, which is seen in the Komodo islands of Indonesia.

So, this is one of the largest reptile like that is almost the size of crocodile or even bigger than the, but land only mainly. So, that is I think around 6 to 7 feet you can read about it. So, this Komodo dragon, they exhibit a feature called parthenogenesis mainly says exhibit this feature called parthenogenesis.

What is parthenogenesis? The female can lay viable eggs without the help of a male, it do not need fertilized egg, like if you have seen many chicken farms you can the female chick will lay eggs without mating with a male, without fertilizing it can lay eggs, but that egg will not hatch, usually it will not hatch it is ok for eating etcetera.

But you cannot hatch it for hatching it has to be fertilized egg. But whereas, Komodo dragon, if they are laying eggs it can hatch and this will give rise to both males and females. Eggs are laid by only female, males will not lay eggs. But the hatch to ones, they can be males and females because many of these reptiles like crocodile or some turtles they follow temperature dependent sex determination.

So, when they lay eggs, they will lay vertically in a pit. So, in summer season the since they laying the eggs are put in a vertical pit, then they will face a gradient of temperature. So, usually in the case of crocodile it is between 31 and 32 or 30 or 32 I have to check it, but around that range between 30 and 32, I think 30.5 to 31.5 something like the very narrow range you will get males. Above 31.5 all females, below 30.5 or 29 something around that range around 30s plus or minus 1 degree you will have males, above and below you will have females.

So, this is called temperature dependent sex determination. Similarly, Komodo dragon also, although the female is laying eggs that is viable eggs that is hatch able eggs that

will give both males and females. So, this is an advantage for Komodo dragon, because it can propagate itself, because it can because otherwise chances of finding a male in the same island is less.

So, a female landed in an island if it could not find a male then chances of propagation is less. Hence it become advantages. Parthenogenesis has become advantages. And now you have both males and females from this offspring and they will follow a sexual breeding. Why I said this because this imprinting ensures nature wants outbreeding, nature do not want pathogenesis, nature want outbreeding mainly because you should be able to incorporate diversity more and more varieties.

So, that the survival of the fittest as per the Darwin's theory of evolution, survival of the fittest can be ensured. How can you ensure the fittest population being formed in a society, in a population. Because you have to bring in changes, not from other species from the same species itself, you need to bring in changes; you need to bring in variety. So, to ensure this you need to indulge in the sexual breeding.

So, the imprinting is a way of ensuring that sexual breeding is assured. Say for example, let us think, a female that is laying eggs and of course, it has to become deployed etcetera by you know retention of the first polar body or second polar body that is a different thing let us do not go into the details of it.

But if a organism is following imprinting a female gene is not going to be expressed in the next generation, only a male copy is supposed to be expressed, then pathogenesis will fall apart that is why many mammalian species or many bird species like I told you chicken. Chicken is laying eggs why it is not able to give rise to a hatchling, mainly because it has many genes that are imprinted and it will not be able to express in the offspring.

So, imprinting make sure that the organism has to make a copy of the new organism only through the sexual breeding; that is, male and female you know mating. So, activity of various this heterochromatic RNAs, shRNA is not limited only to genetic imprinting.

So, they have many other roles, but shRNA plays a major role in the initiation and maintaining of this imprinted status no leakiness is allowed. So, genomic imprinting is a

feature that has to be strictly followed and effectively followed in various organism especially in birds and mammals.

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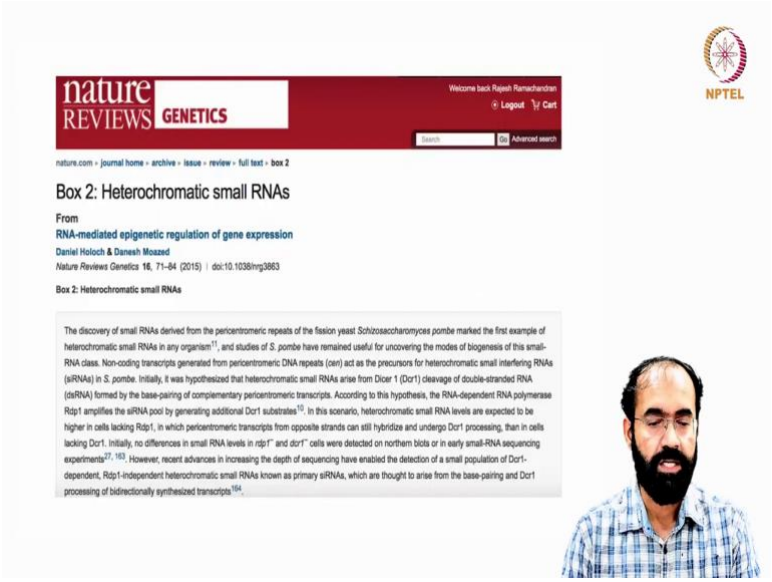

So, you can read more about this heterochromatic small RNAs in this review that is RNA mediated epigenetic regulation of gene expression. It's a quite interesting article and since it is a review you will be able to understand it much better.

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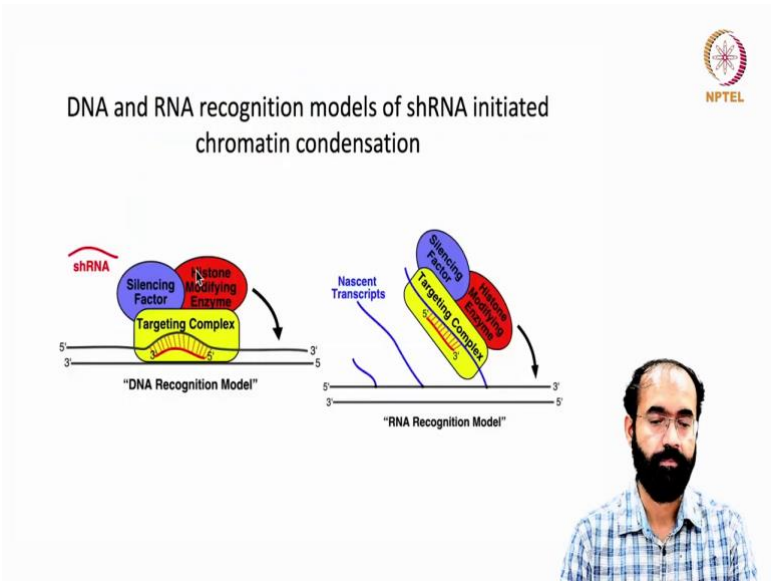

So, DNA and RNA recognition models of shRNA initiated chromatin condensation, means we will in this section we will try to understand, how a shRNA is contributing to

the maintenance of this you know imprinted status. So, we have two models one is a DNA recognition model and an RNA recognition model. We know all genes are located in the DNA and RNA is formed from the specific loci or location in the DNA.

So, you have this DNA and you have this DNA recognition model and this shRNA comes into picture. shRNA has come from a given location of it has its own gene and this shRNA being small it can find a specific sequence like just like how miRNA binding onto the UTR of a given gene. And it will cause its RNA induced to silencing complex and it will cause the repression of those genes.

So, like that this shRNA can find one of the DNA strands and it will pair complementary, it will have match with the complementarity. The other DNA strand is lying empty, you may remember that r-loop hybridization how introns were discovered something similar, it will go and pair. Now, once this pairing has happened, it can bring in other protein factors such as; silencing factors and also histone modifying enzymes.

Once they have occupied that will spread across the neighborhood until there is a boundary is reached. You have seen similar thing happening to the entire X chromosome. Here entire X chromosome is not affected, but a part is affected and in the RNA recognition model, similar thing is happening, but instead of binding onto the DNA it is binding onto the newly formed RNA, which is not completed it is a nascent transcript smaller, longer and longer.

And once this region is exposed this shRNA will come and pair onto the newly formed RNA and it will bring again the silencing factor and histone modifying enzymes and cause the spreading of this silencing events across the neighborhood. Not the entire chromosome it is only in the neighborhood and make sure that this gene is never expressed. So, we will see more in detail about this mechanism of genomic imprinting and more important way of gene regulations in the next class.

Thank you.