RNA Biology
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Lecture - 43
Dosage Compensation and X-Inactivation: Molecular Basis of X Inactivation

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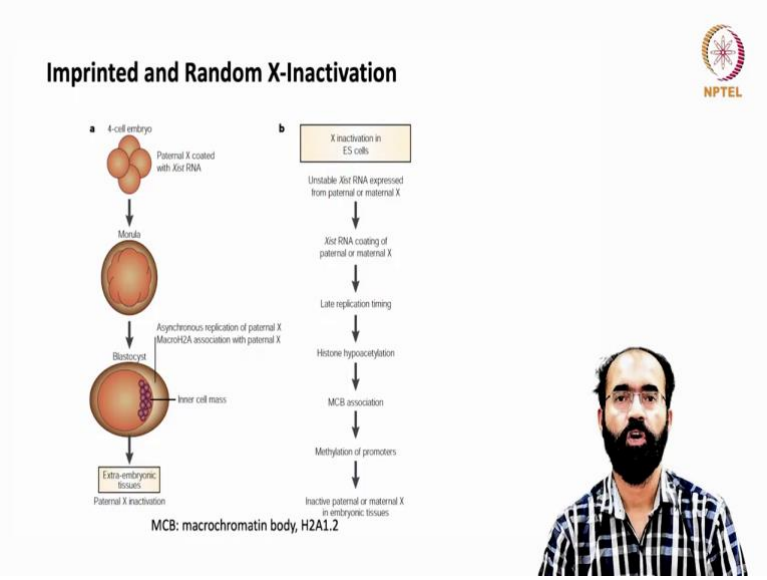

Hello everyone, welcome back to another session of RNA Biology. So, we were here in the previous class that we were discussing the ways in which X chromosome can be inactivated and we saw that in embryos until the formation of extra embryonic layers, the paternal copies inactivated whereas, in embryonic stem cells both the copies either paternal or maternal can be inactivated randomly.

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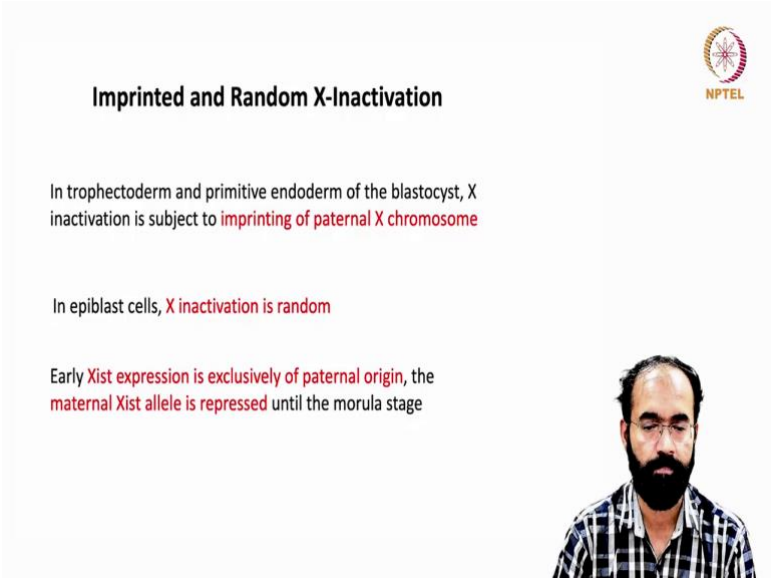

So, what is imprinted and random X-inactivation? In trophectoderm and primitive endoderm, this is embryonic terminology of the blastocyst X-inactivation is subject to imprinting of paternal X chromosome. This is what is called imprinting means there is no equality given to the maternal copy and the paternal copy or here it is biased, biased to inactivate the paternal copy or father's copy until this stage. So, that is why we call it as imprinting, it is been assigned to do so.

In epiblast cell, X inactivation is random and remember epiblast cell is the one which is going to become the embryo, the actual organism. Other part is not going to become the organism, it becomes the extra embryonic layers, other tissues such as you know supporting structures such as placenta.

So, early Xist expression is exclusively of paternal origin, you can guess it why so it is happening, why paternal copy is being arrested because the paternal X chromosome express the Xist and this Xist RNA will code onto the same chromosome and hence it will become inactive.

The maternal Xist allele is repressed, allele means alternative form of a gene like you have every gene two copy, one came from father, one came from mother, but whether both are expressed it depends on which gene, any gene I am not talking about X chromosome, like chromosome number 1, chromosome number 2, chromosome number 3, any chromosome gene. So, if only one copy is expressed, paternal or maternal or

randomly anyone is expressed, we usually call them as mono-allelic gene or such expression is called mono-allelic gene expression.

But In some cases both copies are expressed, paternal also expressed, maternal also expressed, I am not talking about Xist, I am talking about a regular normal gene, any gene; gene A, gene B gene, gene C, gene D, any gene you can like we know 23,000 genes are there in human. So, any of these genes they can either follow a mono-allelic pattern and biallelic pattern.

Many a times this mono-allelic gene expression pattern are called imprinted like what you are seeing here in the case of the X chromosome until the extra embryonic layers the paternal copy express, the Xist hence it is made inactive whereas, the maternal is not maternal copy of Xist is not allowed to express and until the morula stage.

Once it crosses the morula stage the epiblast structure will have a randomization; that means, the maternal one also will start expressing. Now, its a question of time who wins over whom or who answers just like some quiz competition you would have seen that is called you know rapid fire round or bell buzzer rounds like question is free for all the four teams or five teams.

So, whoever ring the bell first will get a chance to answer it. So, this is also something similar to that whichever express the X chromosome will get a chance to get into inactivation of that chromosome

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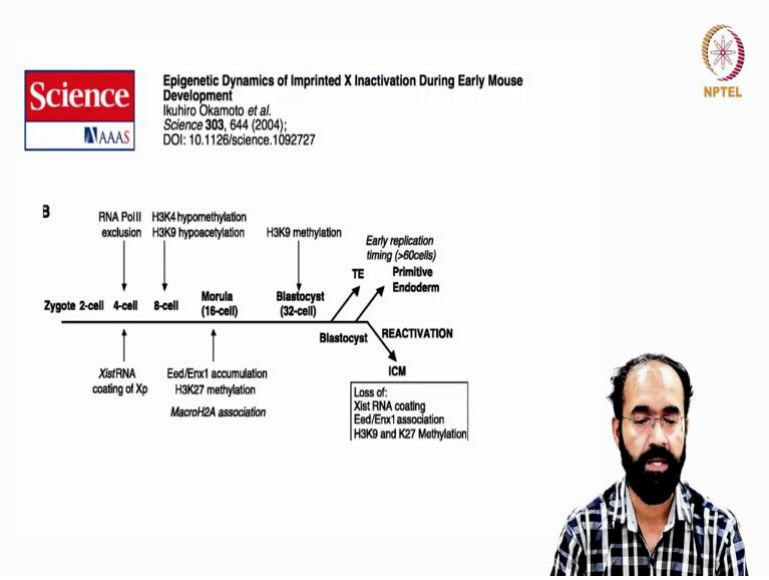

So, this is an article that is published in Science a few years ago very interesting article and Epigenetic Dynamics of Imprinted X-Inactivation During Early Mouse Development. Its published in 2004.

Here they categorically show zygote, it is a single cell stage, it becomes 2-cell, 4-cell, 8-cell and 16-cell stage onwards its morula and blastocyst stage it is around 32-cell stage and we can see that this will give rise to other structures like trophectoderm and also primitive endoderm and inner cell mass. Inner Cell Mass of the blastocyst is called ICM. So, that have the stem cell characteristics.

So, let us see in 4-cell stage RNA polymerase 2 is excluded you do not want any gene to express from the Xist expressing RNA. So, Xist coating happens on XP, XP stands for X paternal, paternal means from father and at 8-cell stage you have H3K4 hypomethylation, H3K9 hypomethylation, hypoacetylation etcetera.

So, H3K4 hypomethylation and H3K9 hypoacetylation takes place at 8-cell stage. Then at morula stage onwards you have EED, ENX, accumulation these are all 2 proteins that accumulate and H3K27 methylation takes place and you have this unique histone protein that is macro H2A association you can see in those X chromosome in in all these cells.

So, this paternal X chromosome start exhibiting these features. At around blastocyst stage you have H3K9 methylation taking place and then you have got TE trophectoderm

and these are all happening at the blastocyst stage and you have early replication timing that is more than 60-cells of the primitive endoderm will show the early replication timing; that means, the replication means cell replication and of course, the X chromosome also have to replicate.

And the reactivation that inactive paternal chromosome is now reactivated in the inner cell mass because if paternal remains inactive then there is no way the maternal will get a chance to get inactivated because both the X chromosome cannot be inactivated. So, you have to start from scratch just like you had a whiteboard or blackboard and you have completely written.

So, now you want to write more you have to erase it completely. So, this reactivation happens in the inner cell mass of the blastula which eventually becomes the proper embryo. So, that Xist can express either maternally or paternally. So, the loss of Xist RNA coating on the paternal X chromosome takes place and EeD, ENX association is lost and H3K9 and H3K27 methylation is lost. So, that the reviving of this paternal X chromosome happens. Now either maternal or paternal can get inactivated.

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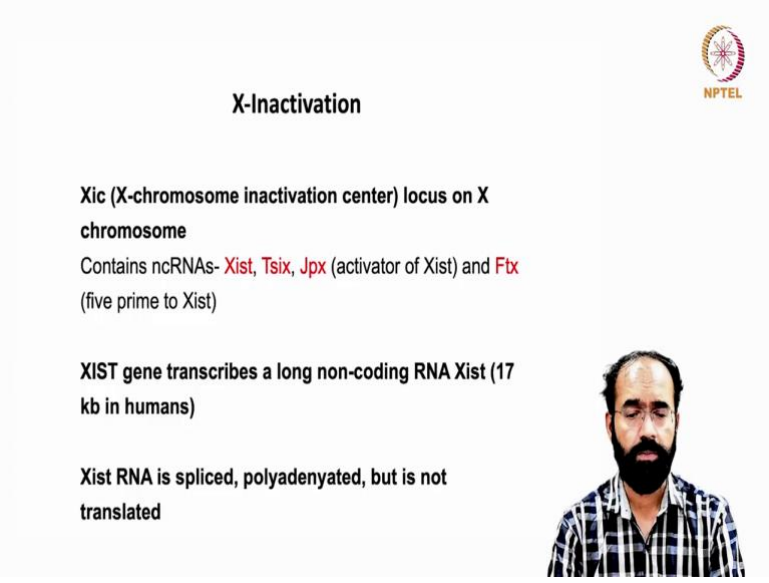

So, let us see X inactivation how does this happen? X inactivation happens via X inactivation centre, X chromosome inactivation centre. So, it happens in a locus of X chromosome. So, it contains several non-coding RNA that is called Xist, Tsix, Jpx and activator of Xist and Ftx and which is five prime to Xist. So, Xist we know X

inactivation specific transcript and we also know Tsix which is the anti-sense strand and Jpx is a non-coding RNA that is called activator of Xist and then Ftx is another non-coding RNA that is five prime to Xist.

So, these are all the main four non-coding RNA found in the X chromosome inactivation centre or XIC and Xist gene transcribes a long non-coding RNA Xist that is roughly 17 kilobases in humans. And Xist RNA is spliced, polyadenylated, but not translated because it do not have the codons. So, splicing and polyadenylation happens before it can code onto the X chromosome.

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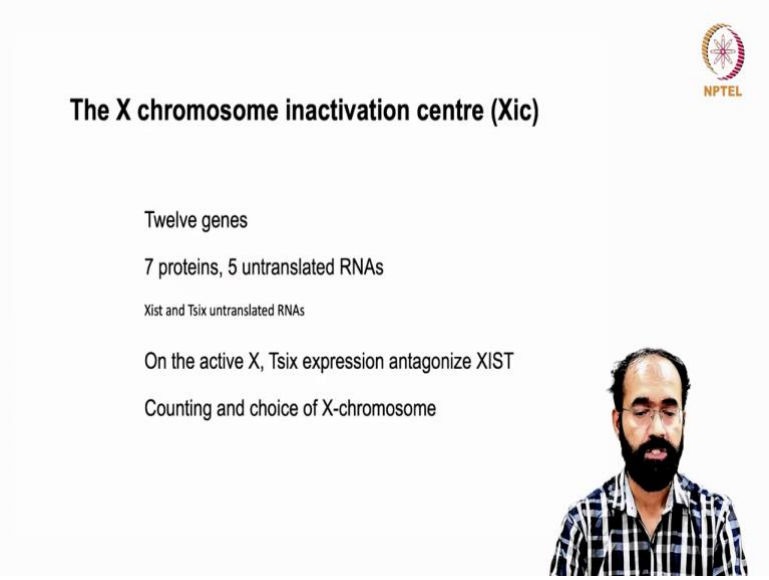

So, X chromosome inactivation centre it has many genes. They are all meant for inactivation purpose. So, roughly around twelve genes are there and it can give rise to 7 proteins and 5 untranslated RNA. Xist and Tsix are one of the 5 untranslated RNAs in the X inactivation centre. On the active X chromosome Tsix expression antagonize Xist; that means, one of them has to express first either Tsix or Xist. If Xist express first then that chromosome will be inactive.

If Tsix express first it will not allow the Xist to act on it. So, also X inactivation centre is important in counting and selecting the choice of the X chromosome. Choice usually is random, there is no preference is given to paternal copy or maternal copy. Choice usually is random, but counting takes place. Counting means cell has to be sure that they have

only 2 X chromosome. What if there are 3 X chromosome? You have to inactivate 2 of them. What if we if we have 4 X chromosome? You have to inactivate 3 of them.

Under any circumstance allow only 1 X chromosome to function. Like I told you in the earlier class that if such a mechanism existed for 21 chromosome, then down syndrome would not have been a problem. What if how nice it would have been if every chromosome pair has an option of counting and making sure only 2 are expressed.

Then down syndrome would not have been a major problem at all. This is not easily possible because every chromosome do not have Xist or similar kind of non-coding RNA or every chromosome is not as small as X chromosome. X chromosome being small, compacting and condensing will be much easy.

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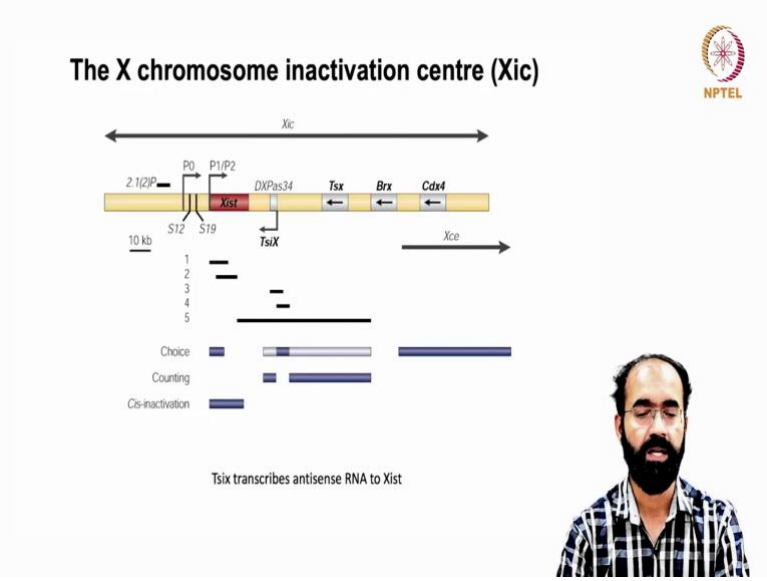

So, huge ones are very difficult to condense with the help of one X inactivation centre it is not that easy. So, let us see in detail the X chromosome inactivation centre Xic. You can see here it is part of the a chromosome itself and this is the scale. So, this much distance counts for around 10 kilobases and the direction of various genes are also shown in arrow.

Direction means which direction it is expressed means 5 prime to 3 prime. So, P stands for P0, P1, P2 these are all different promoters. What sequence elements it is going to

utilize for the expression point of view or means what are the regulatory elements that allow the polymerases RNA polymerases to come and recognize.

So, in Xist you have either P1 or P2 promoter that is expressed the Xist and depending upon which stage of embryonic development you are in accordingly it will use P1 or P2. And then this is going from 5 prime to 3 prime this direction Xist expression and opposite to that you have the other strand opposite direction you have the Tsix. And also, you have got Tsx, Brx, Cdx4 like that few of the non-coding RNAs are there and then you also have Xce.

And their location is been shown with this line like until where it is going. So, the choice has to be made at the level of the Tsix or Xist expression which gene is being expressed is decided randomly and the counting has to take place and counting means how many X chromosomes are there.

Usually, a healthy female will have 2 X chromosome and they have to, but a rare there can be rare situation where 3 X chromosome can be there in that situation both of them two of them will be made inactive only one is made active or allowed to be active. So, that is why counting becomes very important.

So, cis-inactivation is mainly done by Xist whereas, the choice selection also is governed by Xist and also contributed by other downstream genes like Tsx, etcetera. And also, the counting also contributed by some of this chromosome and the choice is also contributed by the Xce non-coding transcript also.

So, this boxes indicate that which genes are contributing to what purpose or what process. So, 3 main things has to be taken care of one is choice which X chromosome should be made inactive and then counting how many X chromosomes are there in a given cell and follow up with the cis inactivation; that means, which chromosome express the Xist that chromosome should be made inactive and which chromosome express Tsix that chromosome should be maintained active.

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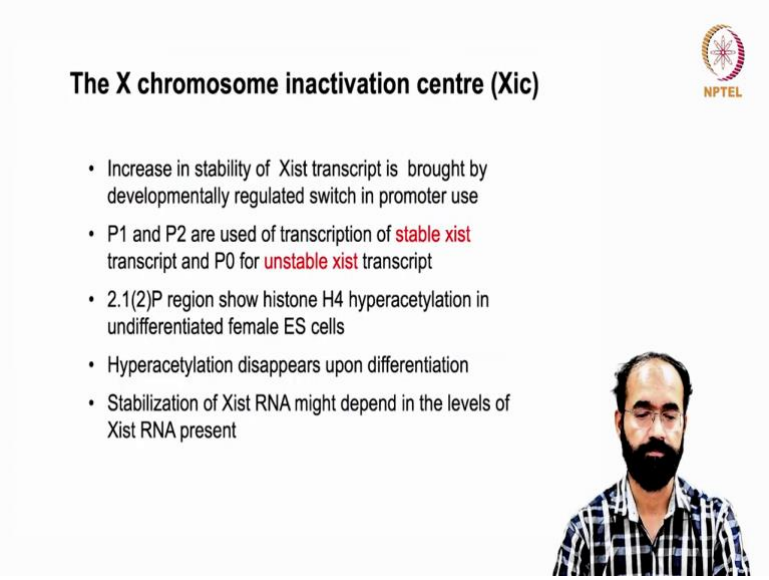

So, X chromosome in activation centre that it can increase in the stability of Xist transcript is usually brought by developmentally regulated switch in the promoter use like we saw P1, P2, P0 like that. P1 and P2 are used for the transcription of stable Xist transcript and P0 is usually meant for transcribing a unstable Xist. So, based on which promoter is being used the stability of Xist can vary.

So, P1 and P2 usually gives a stable Xist, P0 gives an unstable Xist. So, you can guess it. So, the paternal imprinted in activation most likely it will be the P0. Why? Because P0 is not very stable. So, after some time it will be unstable and the once the promoter is off that chromosome can be revived back. But whereas, later stage in the organism's body it has to be P1 or P2. Why? Because we want stable Xist.

So, that chromosome maintains. So, you do not want a weak Xist. 2.1 2 P region shows H4 means histone 4 hyperacetylation in undifferentiated female embryonic stem cell. So, this is a region like P arm and q arm are the two arms on the either side of the centromere of a chromosome. 2.12 region of theP-arm will show a histone 4 hyperacetylation in undifferentiated; undifferentiated means it is maintaining stemness or stem cells a female embryonic stem cell.

And hyperacetylation disappears upon differentiation once the tissue decides to differentiate like stem cells can be forced to differentiate into you know neurons and

other tissues easily by different chemical cues, but they will have this hyperacetylation disappearing once the differentiation kicks in.

Stabilization of Xist RNA might depend on the levels of Xist RNA present. So, amount of Xist RNA also can contribute and decide whether or not an Xist RNA must be stabilized or not. Like you are going to a let us assume a situation like you are going for a war only one person or two person are there you will not be brave enough.

So, your stability can be in question, but if you are going 100 people or 500 people or 10,000 people going then you will have strength in number and automatically it will enhance your chance of survival and stability. So, this happens in the case of Xist RNA also their number also depend the load or the amount present also can contribute to the X Xist stability.

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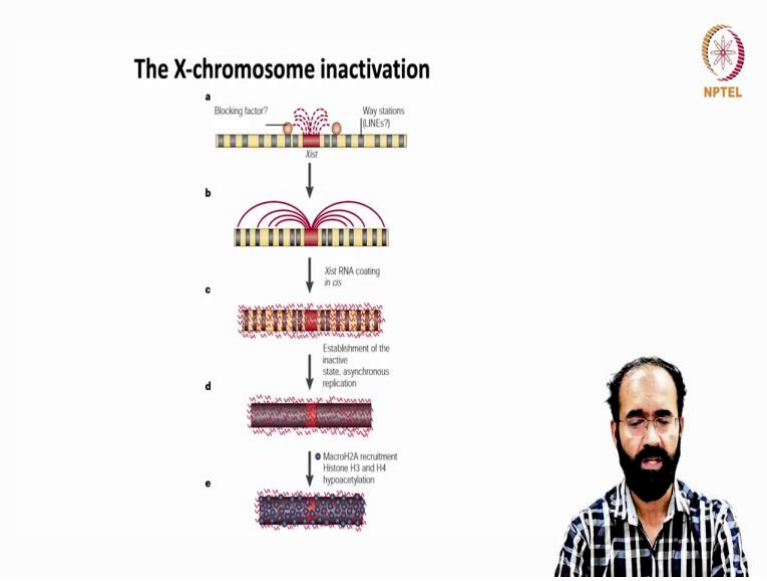

So, now let us see in a cartoonish manner how X chromosome inactivation is taking place. So, this is the X-inactivation centre and the central point is the Xist RNA itself. Once the transcription has started, we know that there is histone modification that kick starts that is you know deacetylation takes place, hypermethylation takes place etcetera. So, this spreads like a nucleation point right from here and they also contributed by certain repeat elements such as lines long interspersed nuclear elements.

So, lines and sines sines stands for short interspersed nuclear elements they are repeats genome repeats their present and they can contribute as stations for spreading this inactivation. So, it is spreading like it is spreading like a wild fire normally in forest if there is wild fire its it does not start from everywhere it starts from a centre and spreads like if you see satellite or aeroplane pictures you will see wild fire spreading like circles to start with and the circle become bigger and bigger and bigger and can spread across.

So, like that it is spreading like this across the entire chromosome and not that this chromosome was longer now it became shorter because of the compaction and Xist RNA start quoting now on to the RNA and which in turn will attract more hetero chromatinizing enzymes that is establishment of inactive state and asynchronous replication takes place.

That means, this X chromosome the DNA of this X chromosome is now asynchronous to the rest of the DNA or rest of the chromosome means they became like out of sync it is not in synchrony with rest of the chromosome. So, it is following a different route it is following very slow replication very late replication etcetera credit goes to this compaction.

And finally, you have the recruitment of this macro H2A histone isoform and histone 3 and histone 4 gets hypo acetylated means deacetylation takes place and that will make the chromatin very tight as you see here.

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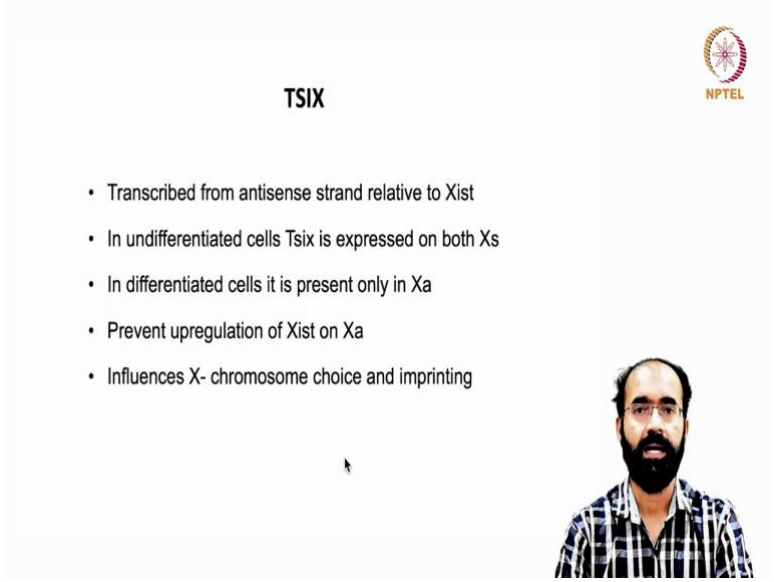

Let us understand more about the Tsix Tsix is transcribed from the antisense strand relative to Xist Xist is in one strand Xist is in the top strand Tsix is in the bottom strand. So, in undifferentiated cells Tsix is expressed in both X chromosomes. So, in undifferentiated cell means it is not committed to become anything. So, Tsix can be expressed from both in differentiated cell it is present only in active X chromosome that is written as Xa.

So, active X chromosome must have strong expression of Tsix to counter even the leaky expression of Xist even if Tsix is expressed that does not mean that Xist cannot express the moment Xist express that also will be vulnerable for compaction. So, Tsix make sure that it do not allow any Xist to coat on to the DNA.

So, it will prevent the upregulation of Xist on active X chromosome Xa. So, Xist should not be expressed in Tsix expressing chromosome and it influences X chromosome choice and imprinting. So, T six also can decide whether or not a given X chromosome should be active or inactive. So, it can decide the choice of chromosome for the imprinting purpose.

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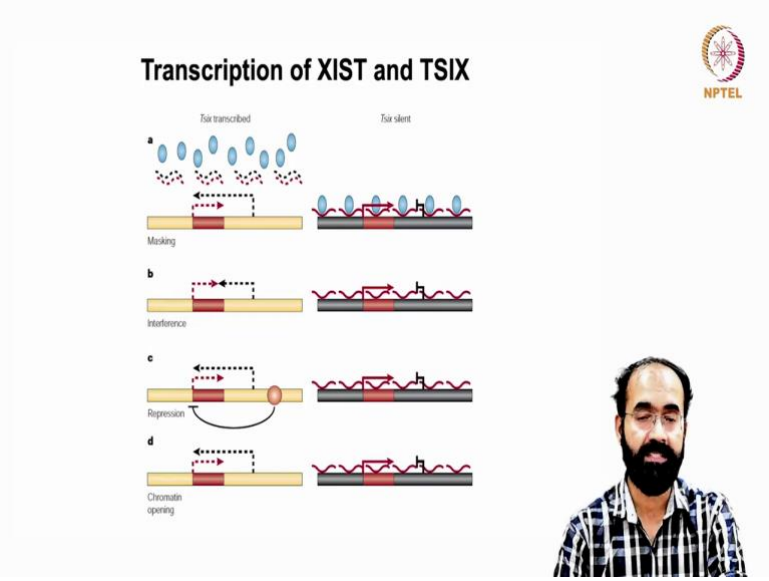

So, let us see how the transcription of Xist and Tsix takes place in a cartoon fashion. So, here you can see Tsix is transcribed and you know the outcome when Tsix is transcribed it can create the masking effect it can cause a masking effect on to Xist and then if Tsix

is expressed it can create an interference remember when the transcription has to happen the transcriptional machinery is assembling on to one chromosome.

So, it can create steric hindrance like you are entering a room and another person also is coming out of the room in a bus rather that will be a better example. Door is smaller one fellow is getting out and you are getting in. So, there will be a stalemate you go neither that fellow can come out nor you can go inside in train bus you can see it.

So, this is called interference it is kind of interacting and of course, here interference means it can pair and it can cause the degradation of both the strands and then comes repression. What is the repression? The Xist is Xist promoter is inhibited by the Tsix. So, this region where the Tsix expression is its regulatory region can inhibit.

So, they can sequester and tighter away all the elements required for you know like you may have seen in some movies etcetera like there may be one shop one small shop where he is selling juice or something and then if a posh shop very nice looking attractive shop comes in the neighborhood then everybody will start going to that new good looking shop the small shop fellow will lose business.

Something similar that there will be more attraction happens to a given promoter the other promoter who is lacking. It is not having enough nutrition in terms of what you call transcription machinery. It will not it will be sequestered by this inhibition and then also by chromatin opening. Whether or not the region responsible for the transcription of Xist is allowed to open.

First of all chromatin has to be removed or chromatin has to be loosened or chromatin has to be remodelled that is called remodelling of chromatin for accessing the DNA for this RNA polymerases. So, that the gene expression can takes place. What if this compaction does not happen you are not allowing the you are not allowing this unpacking at all then also the Xist chromosome cannot express.

So, let us see what are the mechanisms masking can be done by Tsix and interference can be done by Tsix. Repression can be done by Tsix and chromatin opening can be inhibited by Tsix. So, four different mechanisms are possible that can interfere with Xist expression of and vice versa.

You should also think that Tsix is expressed got an upper hand the same mechanism can be done by Xist also because Xist expressing cell should not be able to or Xist expressing cell do not want a Tsix promoter to be expressed active in them because Xist expressing chromosome want to be inactive throughout.

And one more thing is that in a given cell if one paternal or one maternal either of them are inactive it will continue throughout the lineage if that cell is giving rise to two cell later and two become four in their continuously lineage it is not going to be reversed it will continue as long as the organism or that cell is aligned it is not going to get reversed. So, it has to be decided during the early developmental stages itself the randomness.

But it can happen sometimes a given paternal copy or maternal copy expressing cell do not survive for whatsoever reason then what will happen that space will be occupied by the other cell. But this will usually do not happen the way I am saying because once a given X chromosome is inactive all the subsequent cells coming from that particular cell will continue to have the same chromosome maintained in inactive state.

So, maternal is inactive it will stay like that paternal is inactive it will stay like that in that particular cell and its downstream molecules. So, you can also see this cartoon what has been shown here how the mechanism is working in the for the inactivation purpose.

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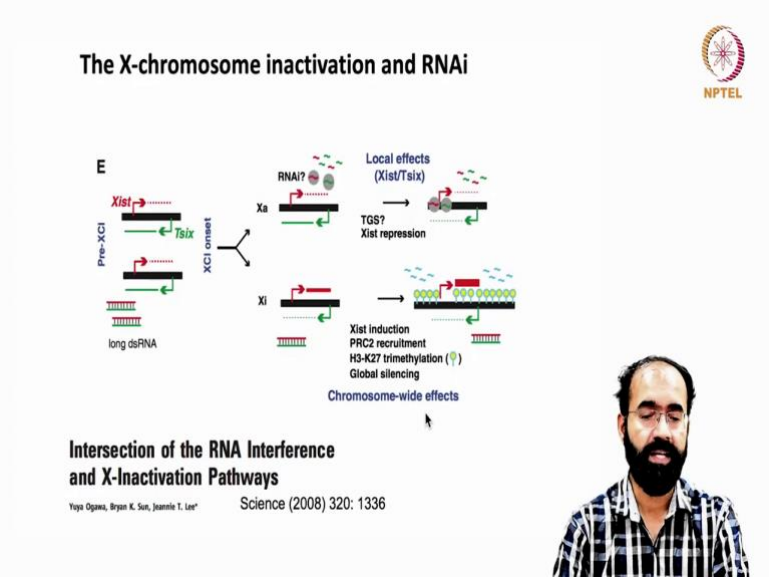

So, we can see how Xist chromosome inactivation and how it is influencing the RNAi. So, X chromosome inactivation is a good example of RNA interference. So, Xist and Tsix can be taken as a typical example of RNA inactivation or RNAi itself.

So, we will study more in detail about the X chromosome inactivation how the RNA interference comes into action in the inactivation process and we also see some pictures images that explain that the actual strength or depth of inactivation with some RNA in situ images in the next class.

Thank you.