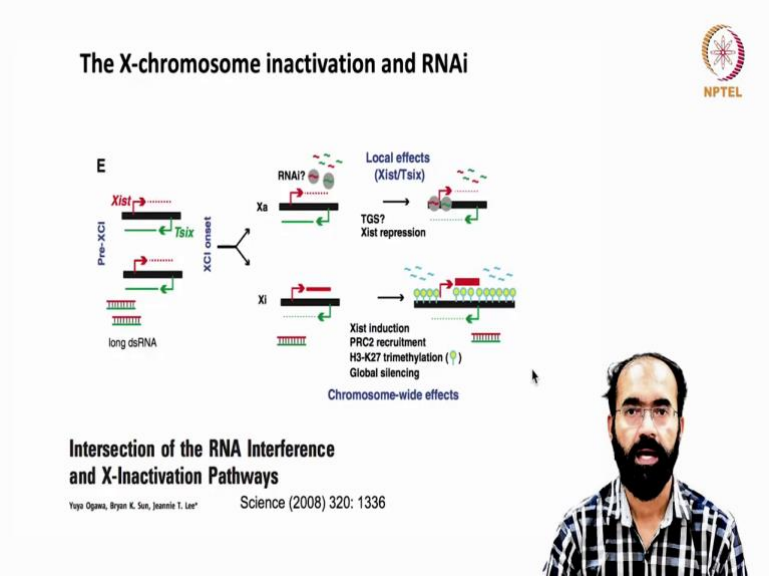
RNA Biology
Prof. Rajesh Ramachandran
Department of Biological Sciences
Indian Institute of Science Education and Research, Mohali

Lecture - 44
Dosage Compensation and X-Inactivation: ES Cells and X-Inactivation

(Refer Slide Time: 00:22)



Hello everyone, welcome back to another session of RNA Biology. So, we were here in the previous class that X-chromosome inactivation and what is its take on RNAi. So, we know Tsix and Xist, they follow exactly the principle of RNA interference and let us dissect it out and this details are taken from this paper published in science that is intersection of RNA interference and X-inactivation pathways.

So, here this figure you can see it the pre X-chromosome inactivation XCI means pre-X chromosome inactivation stage you have a situation that Xist expressed, Tsix expressed and if both are expressed together we can understand that the both will be degraded. That is before the X-chromosome inactivation; that means, both chromosomes are active, because there is no weightage or no bias or no timing bias given to one of them.

Now, let us classify this into two; one is Xa and another is Xi. You will know what is Xa means X active X-chromosome. Xi means X inactive. So, X active we know that Tsix has to be expressed and Xist is not preferred. So, what it should do is it has to create some local effects that is mediated via transcriptional gene silencing and this has to make

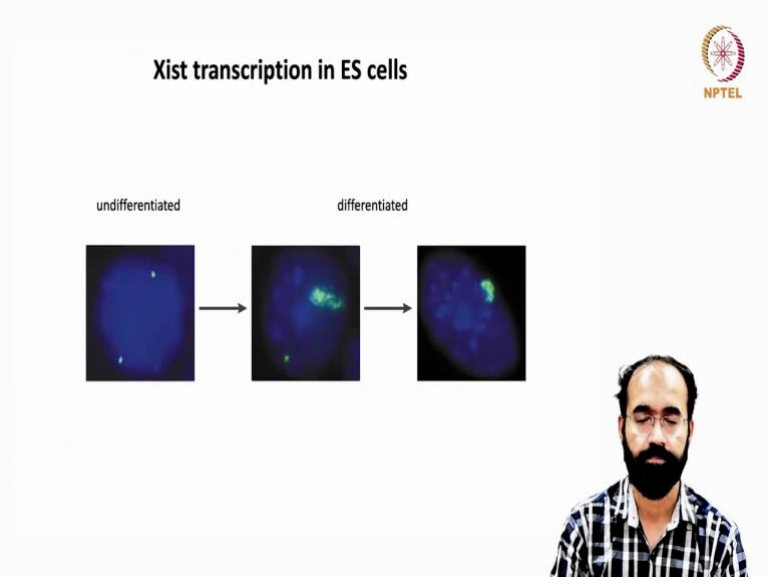
sure the transcriptional gene silencing has to make sure that Xist is not expressing in the Xa means X active.

If TGS is not working on Xist then it cannot remain active it will go automatically inactive. So, the local effects of Xist, Tsix interplay make sure that Xist is not expressed in the active X-chromosome. On the other hand, X inactive what happens? Xist induction happens and PRC2 recruitment of H3K27 trimethylation causes a global silencing and this creates the compaction of the entire chromosome.

That is Xi, X inactive and this will lead to the inhibition of all entire chromosome. So, gene expression will be prevented only the coated Xist stays there. So, Xist induction ensures the global change unlike what you see in the case of Tsix expression. Tsix do not bring in any global change it bothers only the transcriptional gene silencing of Xist. It bothers only the blocking of Xist. It do not bother about the rest of the chromosome.

Whereas, if Xist is expressed it has to make sure that the entire chromosome is off and the coated Xist stays there. So, for entire chromosome in activation it depends PRC2 recruitment and H3K27 trimethylation and triggers a global silencing and rest of them you already know a specific histone isoform, macro H2A recruitment etcetera has to happen.

(Refer Slide Time: 03:51)



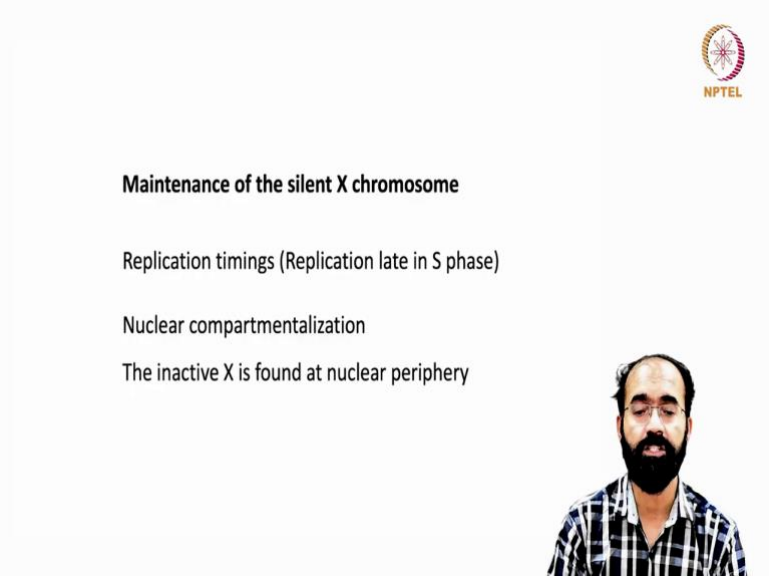
Now, let us see Xist transcription in ES cells, Embryonic Stem cells. So, in undifferentiated cell undifferentiated means it is stem cell remaining as stem cell you can see both 2 dots, 2 dots that is green in color. So, how this is done this technique is called fluorescence insitu hybridization where you take the probe. What is the probe? Xist RNA is the probe you label it with a fluorophore and you hybridize it in a denatured condition onto the chromosomes or onto the nucleus.

So, this entire circle blue color circle is the nucleus and in the nucleus, you can see 2 dots and these 2 dots indicates the there is no weightage of Xist over the other. It is equal, but it is low and you can say low, because looking at the neighbouring pictures. Neighbouring pictures once it is differentiating early stage you can see huge expression of Xist in one of them and you can guess it that is going to be inactive.

Whereas moderate or nil expression in the other chromosome. That dot remains more or less the same. And as the differentiation continues you will see this is disappearing completely, because you do not have even tiniest expression of Xist and the high level of expression continues, because even the tiniest level of Xist expressed that will cause the inactivation even if it is not complete it can cause partial inactivation.

So, you do not want that to happen. So, eventually the one which is having high level of Xist will remain inactive the one with low or nil expression which is supposed to be somewhere here. It is missing. Xist expression is missing means it is an active chromosome. So, this you can prove it by fluorescence insitu hybridization. It is a very powerful technique used in RNA biology.

(Refer Slide Time: 05:50)

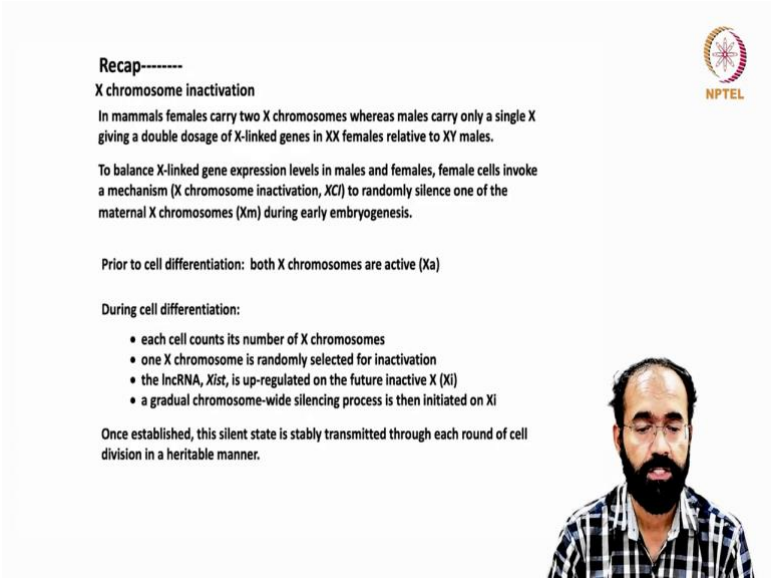


So, maintenance of the silent X-chromosome. So, like I told you in the earlier class also making a 10 storied building is easy means possible, but maintaining that is really tough, because it can go dirty if it is not maintained it can go really bad. Same way once X-chromosome is inactivated it has to be maintained.

So, many things has to be taken into consideration. Such as replication timings that is replication of this inactive X-chromosome happens very late in the S phase of the cell cycle and nuclear compartmentalization. Normally it is compartmentalized in a place where the gene expression events are very low normally towards the periphery of the nucleus gene expression events are very low.

The inactive X is found usually at the periphery of the nuclei where in general the gene expression events are very low. I am not saying that in the periphery there is no gene expression, but in a global way if you want to make as a statement towards the periphery gene expression events are low it is almost like a discarded territory. So, there you will have this X-chromosome inactive X-chromosome located or distributed.

(Refer Slide Time: 07:08)



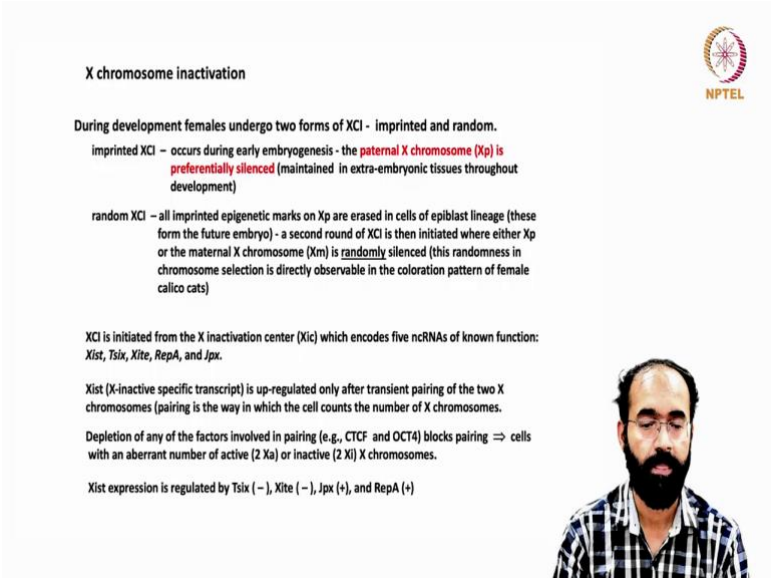
So, let us have a recap of what we have already seen X-chromosome inactivation in mammals. The females carry two X-chromosome whereas, the males carry only a single X giving a double dosage of X-linked genes in XX females relative to XY males. So, this is the principle behind it. To balance the X-linked gene expression levels in males and females female cells invoke a mechanism what is called X-chromosome inactivation or XCI to randomly silence one of the maternal X-chromosome during early embryogenesis.

So, it depends later in the organism either paternal or maternal will be silenced. Prior to cell differentiation both X-chromosomes are made active both X-chromosomes are made active. Now its a question of randomness during cell differentiation each cell counts its number of X-chromosome. How many chromosomes are there in a cell?

If there are three two of them has to be made inactive only one is allowed to be active. One X-chromosome is randomly selected for inactivation the LNC RNA long non-coding RNA Xist is up regulated on the future inactive X-chromosome termed as Xi. A gradual chromosome wide silencing process is then initiated on the Xi. So, that the entire chromosome is now compact.

Once established this silent state is possibly or it is stably transmitted through each round of cell division in a heritable manner; that means, this will continue to be accepted and maintained.

(Refer Slide Time: 09:07)



So, X-chromosome inactivation looking further during development females undergo two forms of X-chromosome inactivation one is imprinted and the another is random. So, imprinted X-chromosome inactivation occurs during early embryogenesis.

The paternal X-chromosome preferred as XP is preferentially silenced and it is maintained in extra embryonic tissues throughout the development. Random XCI happens later on imprinted epigenetic marks on XP are erased; that means, paternal X are erased in cells of epiblast lineage and these form future embryo epiblast cells.

A second round of X-chromosome inactivation is then initiated where either Xp or the maternal X-chromosome that is referred to as XM. Is randomly silenced no biases given to any of the randomly silenced and this randomness in chromosome selection is directly observable in the coloration pattern of female calico cats.

Which we have seen it already examples. That we cannot say that black color is more or yellow color is more or black color is in a particular shape or yellow color is in a particular shape. So, it is absolute randomness no design pattern will be followed. So, it can be random patches you can see.

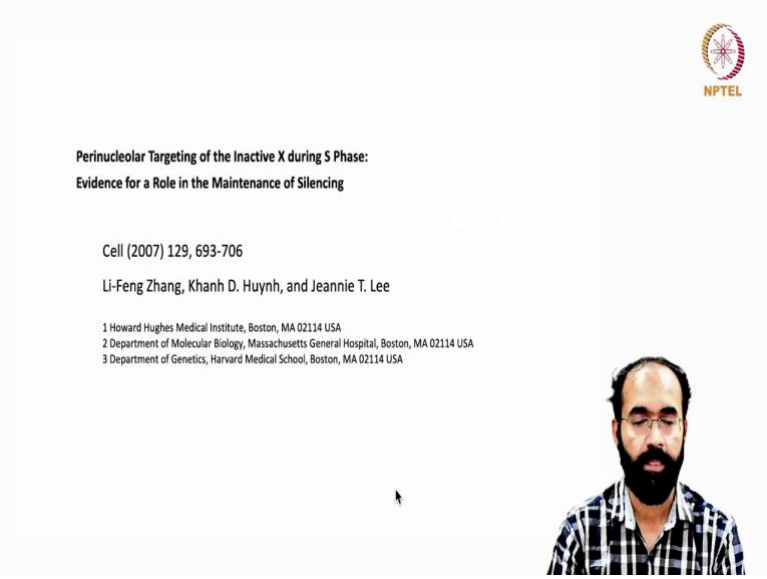
X-chromosome inactivation is initiated from X inactivation center, which encodes five non-coding RNA of non-functions and this non-coding RNA are Xist, Tsix, Xite, RepA and Jpx. So, these are all the non-coding RNAs and Xist is up regulated only after

transient pairing of two X-chromosome. Transient pairing basically meant for counting. And pairing is the way in which the cell counts the number of X-chromosome. So, this is what we should know unless this pairing happens you simply cannot count it.

So, depletion of any of these factors involved in pairing such as CTCF and OCT4 they are important in the counting purpose blocks the pairing of the cells with an aberrant number of active X chromosome; that means, both the X chromosome can remain active or both can remain inactive.

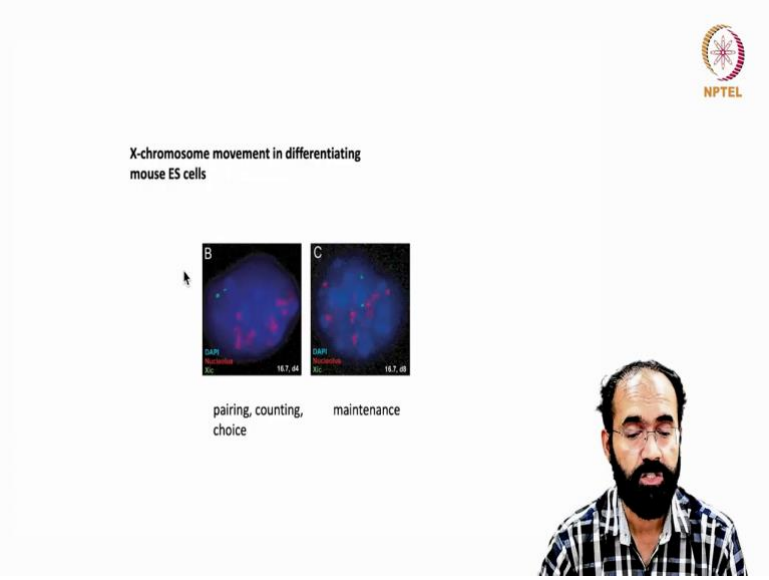
Usually, should not happen if there is a imbalance happens in the expression level of CTCF or OCT4 these are all genes responsible for the counting. Xist expression is regulated by Tsix and in a negative manner and Xite also affected in a negative manner Jpx affects in a positive manner and the RepA affected in a positive manner. So, Xist expression is stringently regulated by various supporting non-coding RNAs.

(Refer Slide Time: 12:14)



So, perinucleolar targeting of the inactive X happens during the late S phase during the S phase and it is maintained in the S phase and it is always found in the periphery and its replication happens in the late S phase. So, evidence for a role in the maintenance of silencing is discussed in this article. So, you can read and we will also address it in a clear manner in the subsequent topics. So, this is a very interesting article. So, which is published in cell few years ago.

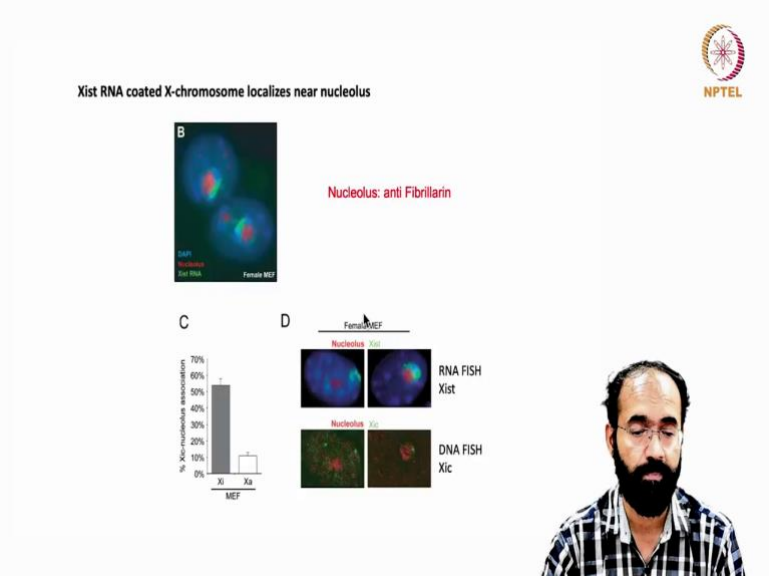
(Refer Slide Time: 12:54)



So, X-chromosome movement in differentiating mouse embryonic stem cell. So, we know that number of X-chromosome during early phase before differentiation happens. Early phase of embryonic development the counting has to happen. Once it is for counting it has to pair for pairing it has to be close by once it is close by then it slowly moves apart.

And so, pairing counting and the choice is decided and two dots are close by you can see here and X inactivation center is what is marked in this green color dot and then they slowly start moving apart and later one of them will start expressing too much of Xist we saw in the previous picture.

(Refer Slide Time: 13:37)

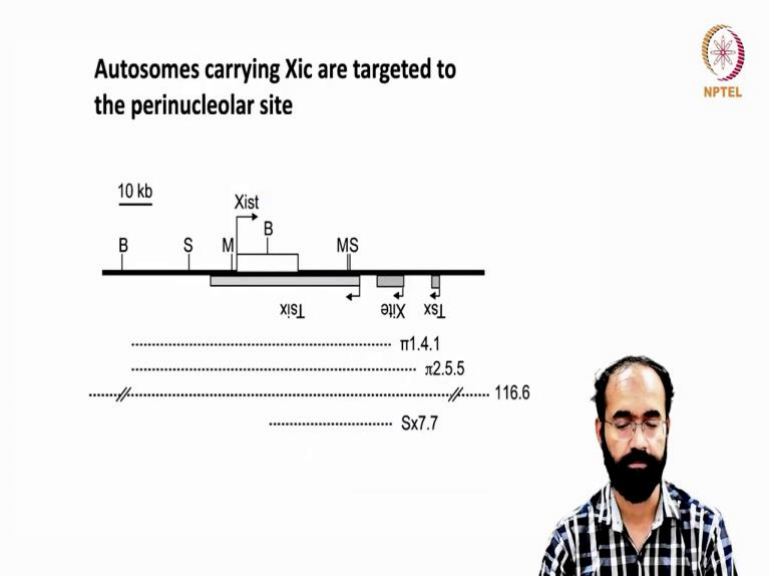


So, what happens the Xist RNA coated on X-chromosome localizes near the nucleolus. So, nucleolus is a place where you have too much of expression of ribosomal RNA. So, this is stained nucleolus is stained with a marker anti Fibrillarin. Staining is done for a protein and that can mark, because nucleolus have got too much of expression of anti Fibrillarin and that is seen in red in color.

You can see the Xist RNA is very much close. So, what it indicates this X-chromosome Xist coated X-chromosome means inactive X-chromosome it comes closer to the nucleolus which in turn eventually become closer to the periphery of the say like here it is closer to the nucleolus then it eventually located to the periphery of the nucleus. This is like a circular shape like a oval shape circular and it is moving towards the periphery.

And whether you do RNA fish or DNA fish like fish stands for fluorescent in situ hybridization you can use RNA as a probe or you can use DNA as a probe in both the cases you can see that its location is towards the periphery. So, X inactivation center always have got a nucleolar association if it is inactive X-chromosome and if it is an active X-chromosome, it is not having a nucleolar association. So, this is something important to notice.

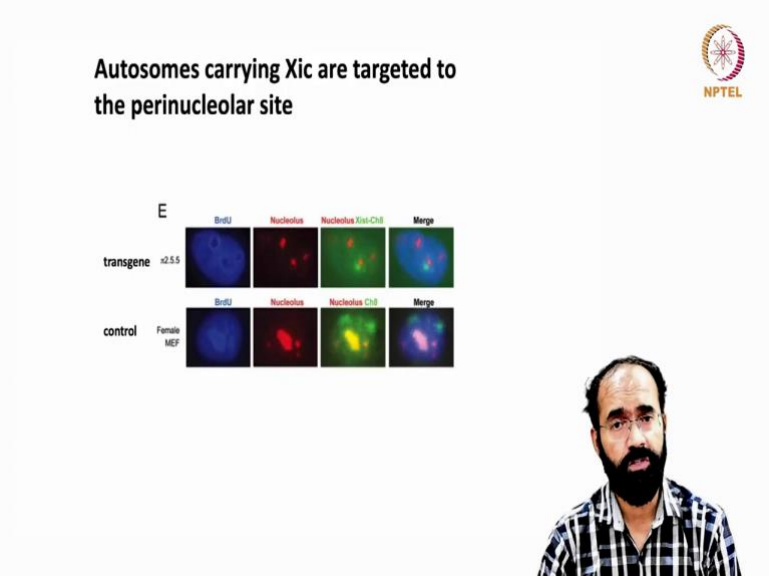
(Refer Slide Time: 15:13)



So, autosomes carry carrying the X inactivation center are target to perinucleolar site. So, if you are taking this X inactivation center that portion you are taking it from the X-chromosome and you are inserting it onto a another chromosome which is autosome. Autosome means chromosome number 1 to in case of human chromosome number 1 to chromosome number 22 is called autosomes.

So, you take this X inactivation center and put on to a any other autosomes you see that this chromosome now go closer to perinucleolar site. So, this indicates X inactivation center have something to do with association with the nucleolus. So, you can see here this different location this is inactivation center Xist, Tsix and you have Xi Tsx and many more genes are located. So, this center this region is what you are detaching and implanting onto a autosome.

(Refer Slide Time: 16:21)



So, autosomes carrying X inactivation center are targeted to perinucleolar site. You can see this in this example. So, bottom panel is the control; that means, they have not received any modification. X inactivation center is untouched whereas, in transgene you have X inactivation center inserted onto some autosome.

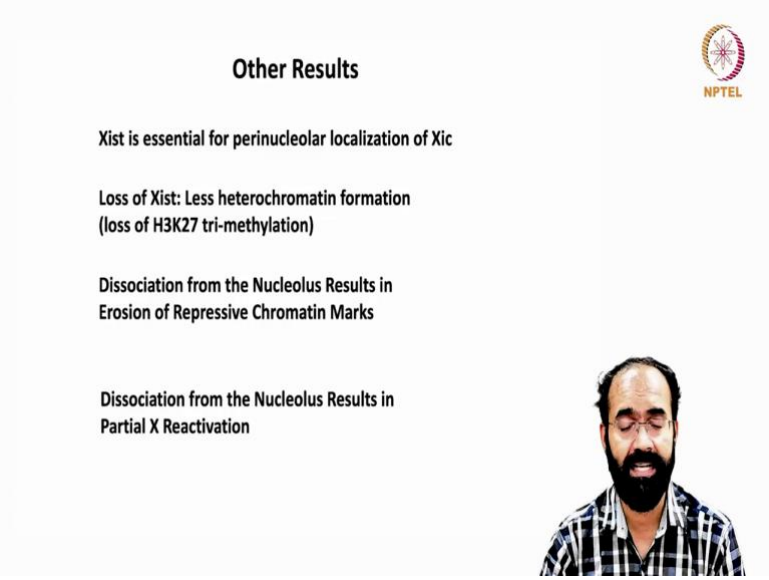
You can see here this is BrdU, this is a nucleolus training red color and then you have got Xist. Now, it is attached onto chromosome number 8 Xist in this experiment whereas, in the bottom panel chromosome number 8 is normal it is not been done with anything. So, wherever you say red color dot that indicates the nucleolus and wherever you see this green color dot that indicates X inactivation specific transcript is present.

So, once you merge them you realize whenever you put Xist or X inactivation center totally in chromosome number 8 it has got a perinucleolar association. But no such association you can see if you have a marker for chromosome number 8 both the chromosomes are seen properly. Here remember here in this control the chromosome number 8 is not tagged with X inactivation center.

Hence it does not have X inactivation specific transcript, but it has some marker for chromosome number 8 that some other gene expressed specifically to chromosome number 8. So, what you should understand chromosome number 8 do not have an affinity for nucleolus, but the moment you put X inactivation center in chromosome number 8 you will have its affinity going closer to the nucleolus.

So, what it tells you that during the inactivation process the X-chromosome which normally have the actual Xist or actual Xic X inactivation center will associated with nucleolus is not by randomness the credit goes to the X inactivation center itself. So, and this might be contributing for the peripheral localization eventual peripheral localization of the X chromosome.

(Refer Slide Time: 18:36)



So, let us see other supporting results. Xist is essential for perinucleolar localization of X inactivation center. Loss of Xist less heterochromatin formation means you will not have H3K27 tri-methylation effectively. So, what it indicates that X inactivation the guide or the inspiring force of X inactivation center is Xist itself.

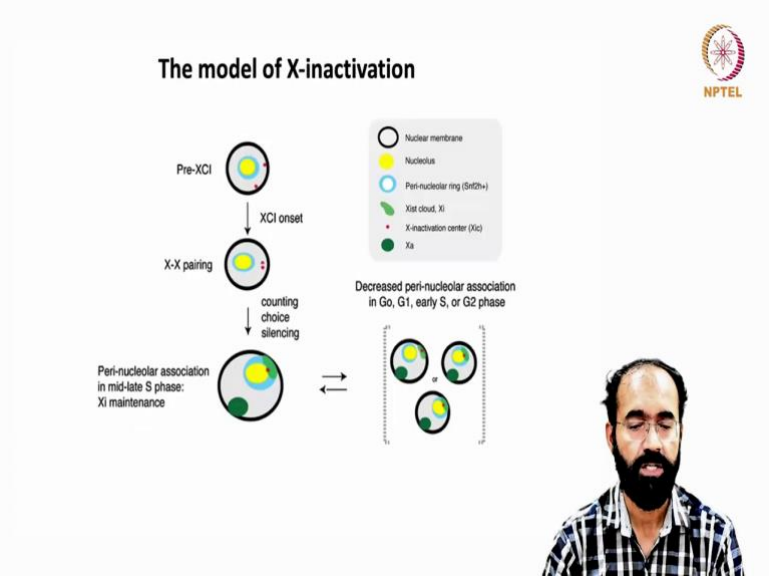
So, dissociation from the nucleolus results in the erosion of repressive chromatin marks. So, what researchers have shown that if you try to disassemble. That let this X chromosome inactive X chromosome be not allowed to interact with nucleolus then the maintaining becomes little difficult. It just like a newborn baby is separated from the mother. Of course, it may survive, but it will not survive as effectively as a newborn baby survives with the mother.

Same way if you prevent the nucleolar association then the inactive X-chromosome need not stay inactive. It is vulnerable to get active, vulnerable to get into active phase. So, dissociation from the nucleolus results in partial X reactivation. So, what it indicates?

The inactive X-chromosomes association with the nucleolus is not by chance, but an obligation it is necessary as a requirement it is interacting.

And if you prevent that interaction by you know chemical tools or by you know genetic tools you can make the X-chromosome inactive X-chromosome to reactivate partially.

(Refer Slide Time: 20:23)



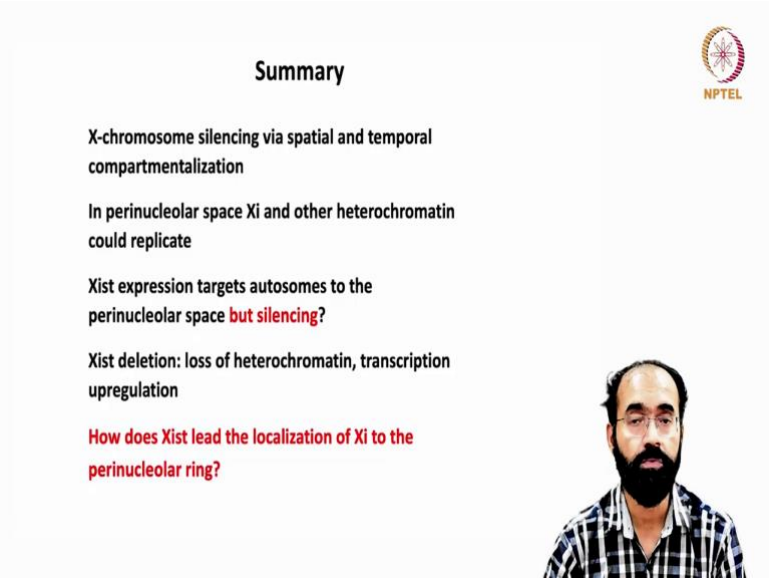
So, let us see in this cartoon a model for X inactivation. So, you have pre-X-chromosome inactivation; that means, both X-chromosomes are given a equal strength equal bias to express that is pre-X-chromosome inactivation. Once X-chromosome inactivation is on set then there has to be a pairing like you can hear they are separately present. This blue color and yellow color dots are nucleus and nucleolus and they are now counting.

They are paired now, X-X pairing is happening and there counting happens and then the choice of silencing also happens. Then what happens? The perinucleolar association in the mid-late S phase takes place and this has to be occurring or this has to be preferred for maintaining the X-chromosome inactivation.

If nuclear association is disassembled then maintaining the X-chromosome in inactive state can be problematic. So, this is the key that nuclear membrane black color and blue color is the nucleus and the yellow color is the nucleolus and this green color dots are Xist and also inactive X-chromosome and red color dot is the X inactivation center and the green color oval shape is Xa.

So, what happens later is decreased perinucleolar association in G0, G1 and early S phase or G2 phase can be seen. So, eventually they will have decreased perinucleolar association, because this is required for the division process for the you know for the further replication, because even an inactive X-chromosome it has to be allowed to divide because it has to unpack by late S phase it has to be.

(Refer Slide Time: 22:37)

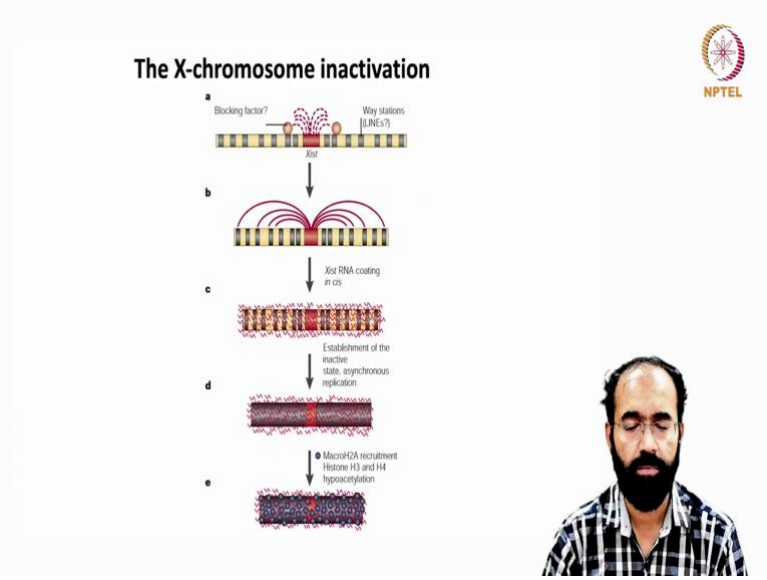


Allowed to it has to be given a choice to divide So, to summarize the X-chromosome silencing via spatial and temporal compartmentalization it is not just Xist alone is required it also has to merge or colocalize or go to the vicinity of the nucleolus to maintain that.

In perinucleolar space X inactive means Xi or inactive X-chromosome and other heterochromatin could replicate that is a place where they replicate and Xist expression targets autosomes to the perinucleolar space, but they may not be getting silenced as it is occurring with the X-chromosome in at least in that experiment where they tried chromosome number 8.

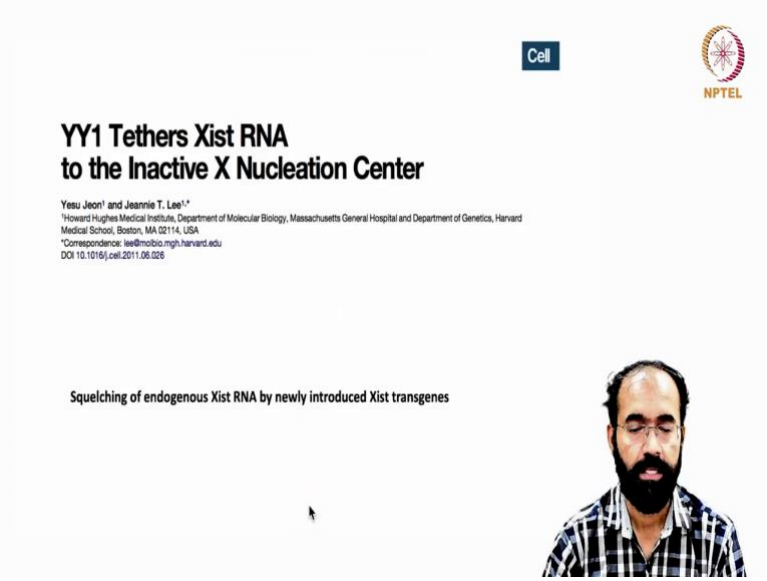
Xist deletion what happens loss of heterochromatin transcription up regulation is seen if you delete the Xist. So, how does Xist lead to the localization of X inactive or inactive X-chromosome to the perinucleolar ring? So, this remains like a big question.

(Refer Slide Time: 23:47)



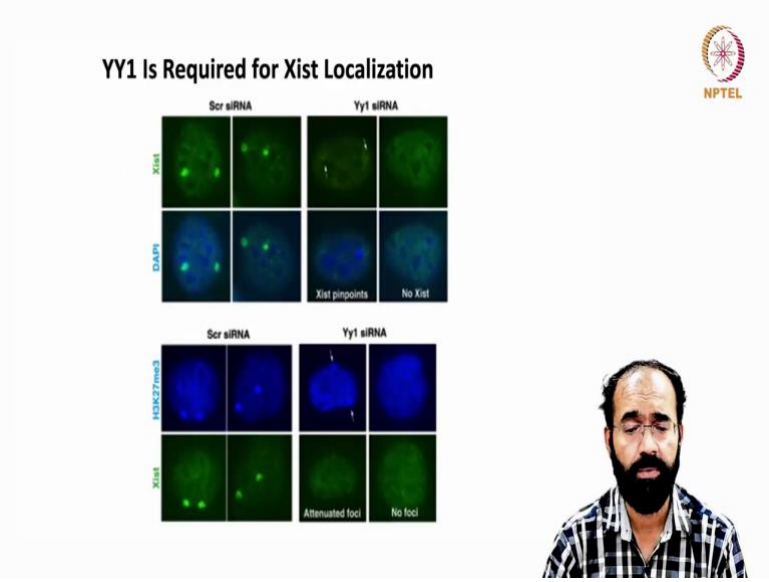
So, this picture we have seen it already. So, let us see how X-chromosome causes the inactivation in a Xi the Xist is expressed from the Xn activation center and it spreads from the nucleus point and it brings down it causes the condensation brings down these line elements closer and Xist RNA codes the entire chromosome and it brings a establishment of a inactive state and it also finally, recruits specific macro H2A recruitment the specific type of histone and it seals or finalize the compaction.

(Refer Slide Time: 24:32)



Now, let us see another article which discussed about the role of a RNA that is role of this Xist RNA tethered onto the X inactivation center by a protein called yin yang. That is called YY1. So, YY1 is capable of tethering this inactive Xist RNA or the for causing the X-chromosome into inactive phase. So, Xist RNA need to be coated some glue someone has to stick this Xist onto the X inactivation center. So, this is facilitated by a protein called YY1.

(Refer Slide Time: 25:18)



So, how can we prove it YY1 is required for the Xist localization. So, we can see Xist expression in green color this is a RNA in situ hybridization and you also have a situation this is scrambled siRNA. In this whole set and here you have got YY1 specific siRNA. What is siRNA? We know that it is used for blocking the expression that is called gene knock down.

So, if you use siRNA against any gene that particular gene production or the particular RNA translation is prevented. Why? Because siRNA will cause the degradation of that mRNA. So, you will not have the protein. So, in this experiment you have scrambled siRNA; that means, it is not targeting any mRNA it is random whereas, this is targeting the YY1 mRNA as a result the YY1 protein is not formed. So, in this section and the bottom panel is the DAPI that marks the nucleus. So, blue color one is marking the nucleus.

So, you can see when you use scrambled siRNA you are having a H3K27 methylation present normally whereas, if you have YY1 siRNA you do not have you lose this H3K27 methylation and Xist staining if you are doing in scrambled siRNA if you have you have both Xist that is present. But if you get rid of the YY1 in the YY1 siRNA one you do not have the Xist RNA present.

So, what indicates Xist RNA to go to the center you need to have the YY1 RNA. So, we will go through this importance of YY1 in localizing the Xist onto X inactivation center more in detail in the coming classes.

Thank you.