RNA Biology
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Lecture - 41
Dosage Compensation and X-Inactivation: Dosage Compensation of X

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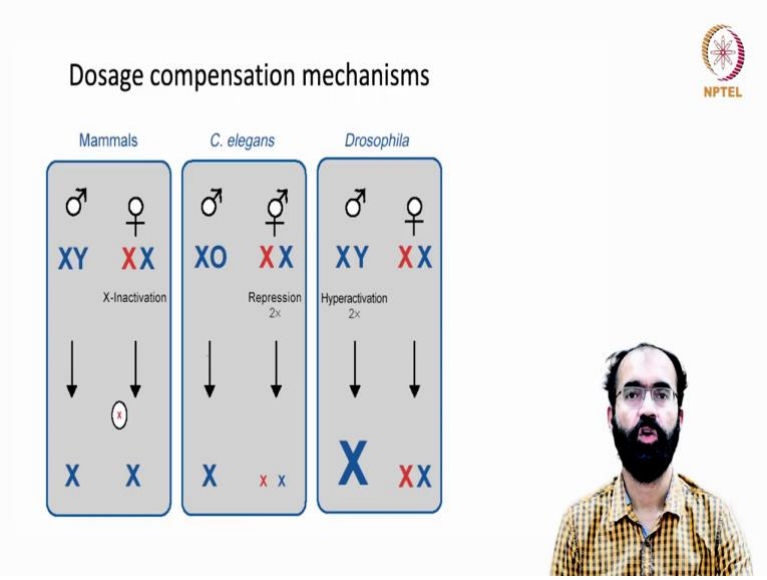

Hello everyone, welcome back to another session of RNA biology. So, we were here in the previous class that we were discussing about the different dosage compensation mechanisms. So, a given cell will have all sets of somatic pairs of chromosome normal. They have no disparity, but when it comes to sex chromosome, it need to match either to that of a female or to that of a male to have uniformity across both the genders.

So, the matching or the compensation is occurring across the genders. So, that the rest of the chromosome, how in evolution a given dose of a given protein is supposed to work so and so. It just like I gave an example of number of tires and number of engines you are making; it should have a matching.

So, if there is a mismatch, number of tires produced to grown up or number of engines produced to grown up can have a mismatching. So, to balance it out just because one of the gender have got a two car manufacturing factory, it cannot keep producing one of the products more.

So, to compensate that you will either reduce the production capacity of that factory or increase the other say tire or engine whichever was compromised, its increase the production capacity of that to match it up. So, this is what usually happens in various organisms.

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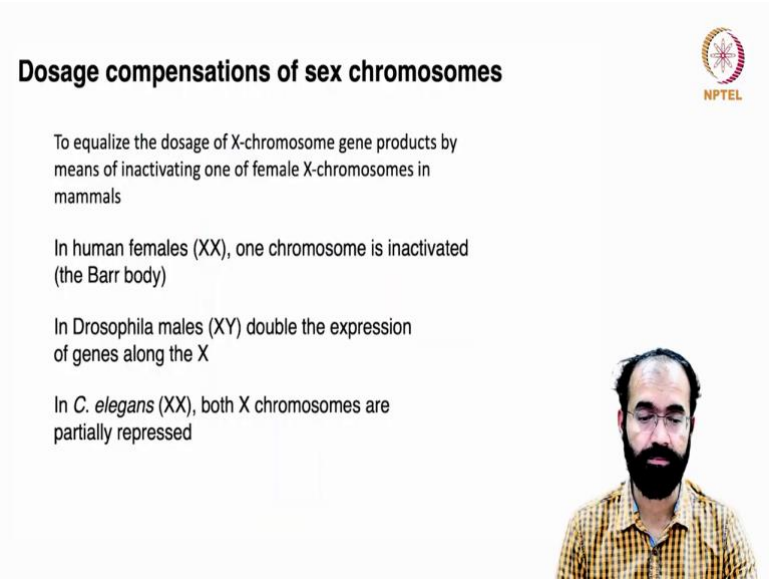

So, dosage compensation of sex chromosomes mainly done to equalize the dosage of X-chromosome gene products that is proteins what are produced and also many RNA genes and the gene products by means of inactivating one of female X-chromosomes in mammals. So, this is the fundamental principle.

So, in human females the XX bearing human females, normal human females will have 2 X-chromosomes. One chromosome is inactivated and that is called Barr body that can be seen under a light microscope. If you take a cell and stain, it and see if you see a highly dense or a highly pigmented body in the nucleus that is most likely a Barr body and that indicates that particular cell is from a female.

In drosophila males XY that they double the expression of the genes along the X-chromosome to match that of the female. In a C. elegans XX both X-chromosomes are partially repressed like we discussed so far.

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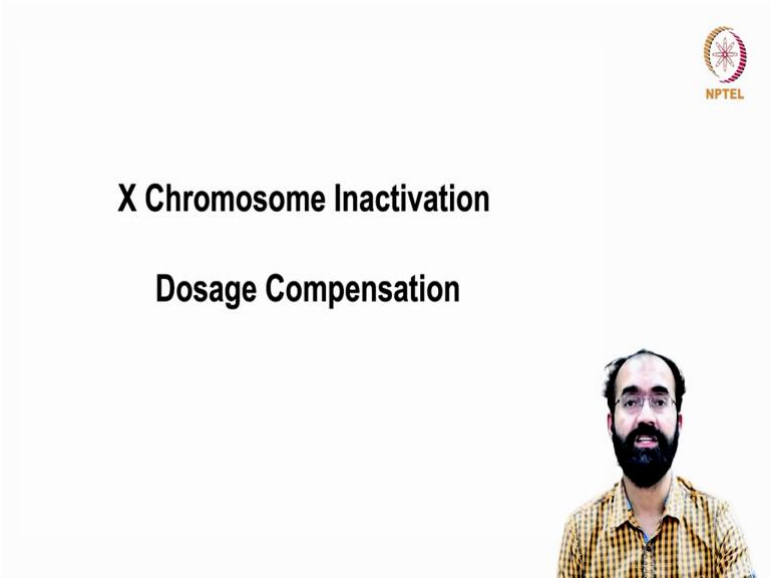

So, X-chromosome inactivation or dosage compensation is a vast research topic and lots of research go on this area and lots of research happen across the globe on the inactivation mechanism. Although we know the principle behind it. We do not fully understand the exact mechanism of it, because it is quite deep and dense research topic the x chromosome inactivation in mammals.

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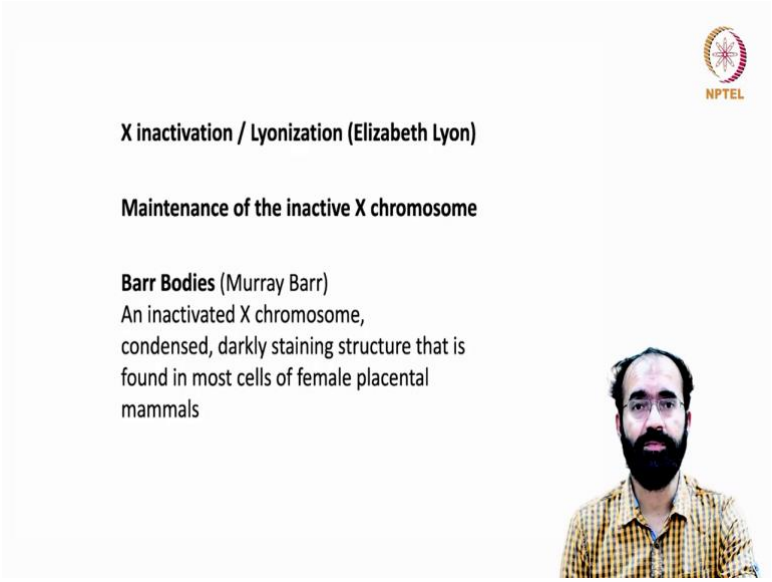

So, X inactivation is also called lyonization. After the scientist Elizabeth Lyon who discovered it. So, it is meant for the maintenance of the inactive X-chromosome. So, it

has to not only cause the inactivation of the X-chromosome, but you have to maintain it also. It is just like you made a 10-storied building, but there is no maintenance. What will happen after say couple of years or at least 10 years it will become like a forest nobody can leave there.

So, inactivation also its like that if inactivation is triggered it has to be maintained also. So, that is not an easy task. If any mismatch happens or any failure happens to that inactivation then the dosage compensation is fallen apart then that can lead to lots of problems something similar to that you know how down syndrome affects.

Because chromosome 21 trisomy; that means, instead of 2 copy you have 3 copy that is the major cause developmental deformity, kind of retardation. Some cases it is very severe that they may not be talking properly etcetera.

So, down syndrome is a problematic scenario simply because one extra chromosome and actually if it was a proper thing to happen then it could have been addressed by dosage compensation, but down syndrome is a problematic situation the organism has not experienced such a thing and the system do not have an option of negating it or nullifying it.

Unlike the X-chromosome inactivation you saw in females. If X-chromosome inactivation do not happen then it can lead to lots of lots of problems. And some examples we will see you can artificially create a female with 4 X-chromosome then also all the 3 will be inactivated. You can make 3 X-chromosome then 2 of them will be inactivated. So, the system knows a counting that how many X-chromosomes are there and make sure that only one is turned on. Such a mechanism do not function in the case of down syndrome.

So, many researchers try to use the same strategy of inactivating a down syndrome extra 21 chromosome similar to what is occurring in the dosage compensation of X-chromosome, but partial success partial results are there, but human subjects the implementation becomes a challenge, because you are talking about a developmental stage onwards you cannot handle or you cannot interfere with a you know genetic possibility.

Of course, human a gene therapies kind of approved people some countries are doing it, but it has never reached a stage where you can intervene right from the boom stage onwards, but people have shown that there are some ways of inactivating this extra 21 chromosome in down syndrome patients and can give some promising result all cell line studies mainly.

So, but it is not a practical option yet to cure the down syndrome. So, they have another name that is a lyonization is a mechanism after the Elizabeth Lyon and they are often referred to as this inactive X-chromosomes seen in female cells are referred to as Barr bodies and after the scientist Murray Barr who discovered it. And inactivated X-chromosome is what is called Barr body. And it is a condensed darkly staining structure that is found in most cells of female placental mammals.

Placental mammals are a group of animals that have a placenta during the development. Some animals do not have placenta they are called non-placental mammals. So, we will not go into the you know embryology or animal classification, but understand placental mammals are the one which follow this kind of X-chromosome inactivation.

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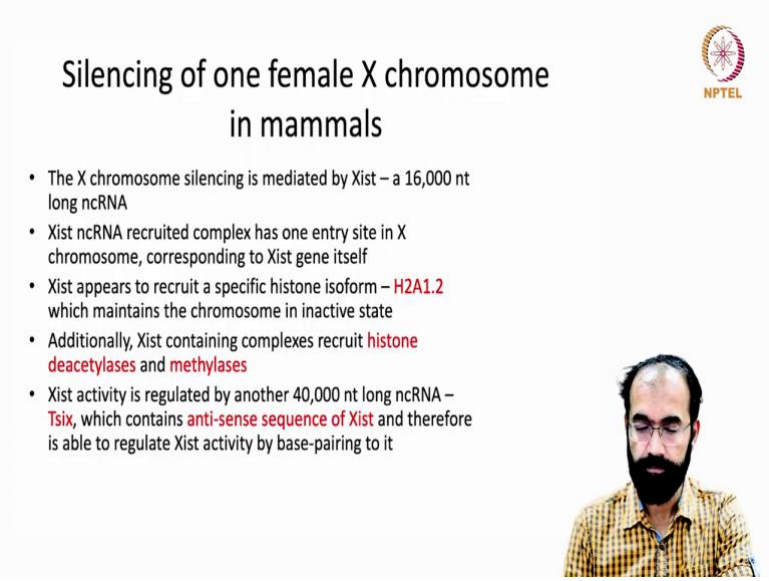

So, silencing of one female X-chromosome in mammals takes place during the very beginning of the development itself. The X-chromosome silencing is mediated by a large non coding RNA called as Xist, Xist what is Xist? X inactivation specific transcript. So,

it's a short form of that long word that is Xist. Which is around 16000 nucleotide long non coding RNA. So, naturally it is considered as a lnc, long non-coding RNA.

So, Xist known coding RNA recruited and this complex has one entry site in X-chromosome corresponding to the Xist gene itself. So, how does this function? Xist gene is present in the X-chromosome. Once this gene is expressing it will give rise to Xist transcript and this Xist transcript go back and attach on to the same place where it was produced and this is the simplistic way of explaining how the inactivation takes place. Of course, this is the only the beginning actual inactivation has much more to it.

So, Xist appears to recruit a specific histone isoform. What are histones? Histones are proteins that interact with the DNA and to compact like all of you have heard about chromosomes, right. So, in a Meta-phase stage of cell division you see really fat chromosome. Actually, chromosomes are nothing, but DNA.

But during the cell division this DNA is compacted very strongly with the help of histones and these histones protect the DNA and also compact the DNA. Both way it helps and of course, histones have got lot of role in the gene expressions also. There is something called histone code you may have heard about epigenome, epigenetics etcetera.

So, histones and their acetylation, methylation etcetera at a specific lysine residues can decide whether or not a given gene should be expressed at a given time. But for this inactivation the Xist RNA once it is coated on to the same place where the Xist gene is located it will recruit a specific histone isoform that is H2A1.2. So, this is a unique histone isoform which is seen only in the inactive X-chromosome which maintains the chromosome in its inactive state.

Normal chromosomes do not use this isoform. It is only used for the inactive x chromosome. Additionally, Xist containing complex recruit histone, deacetylase and methylase. What is histone, deacetylase? If a histone is acetylated, histone can have acetyl group attached on to them. So, this will make the histone less attracting towards the DNA.

Normally histones have lots of basic protein basic amino acids. So, histone by and large is basic in nature. What is DNA? DNA is acidic in nature. So, if DNA is acidic, histone

is basic they will have attraction towards each other. And they will form real compact chromosome structure or chromatin structure.

Now, if you can add acetyl group to specific basic residues not any random amino acid residues, some basic residues such as lysine, lysine is a basic amino acid. So, if you add basic means alkaline in nature. So, basic base and acid you may have heard about it or you know about it. So, basic means do not think that it is I am not talking about fundamental like basic principle not like that. I am talking about the alkalinity.

So, the lysine is alkaline or basic in nature that if it undergoes acetylation then it is no more that much basic, because now it had has gotten acetyl group. Like if you have 1 glass of milk and you add 1 glass of water or 1 litre of water, then it is no more that strong right it became diluted. So, acetylation dilutes the alkalinity or basic nature of the histone.

So, if acetyl groups are added to it that chromatin becomes loose and that DNA is loose from this histone protein that is helpful when you want to have gene expression, because we know gene expression RNA transcription takes place from the DNA. And if DNA is covered in histone, how are you going to touch the double stranding double stranded DNA, then they need to be opened it up.

So, acetylation is very helpful when you want to loosen the chromatin. So, that the DNA can be accessed by the RNA polymerase etcetera. So, what happens here is Xist once it is bound down to this chromosome it recruits histone deacetylase. What is deacetylase? It will remove the acetyl group. Even if there is a here and there one or two acetyl group attached on to the histone that will make the chromatin or the chromosome loose packing loose.

Just like you may have done in your childhood that you collect the post-stamp and if you have a stamp collection hobby what you do you apply little bit of water or some people put little bit of saliva and some liquid you put it then the glue become loose and loose and loose and; So, that the stamp can be easily removed.

Same logic applies of acetylation is nothing, but pouring water onto a stamp in an envelope. So, that you can remove the stamp without damaging you are diluting the glue

you are making the glue loose same logic applies when you are having the acetyl group attached on to it.

The opposite happens if you are drying the envelope. See you applied some water say normally you fix the stamp onto an envelope. You will not pour water onto it you will dry it. Why you dry it? Because you want the stamp to be holding tight. So, this mechanism happens if you deacetylate it. So, removing the acetyl group make the compaction of the chromatin and other group are there which does the same role that is a methylation if a histone is methylated it becomes less loose it become compact.

So, methylation will happen deacetylation also will happen it is just like you know I am pulling you and someone is pushing you from the back. So, that definitely you will fall down. So, if one fellow is pulling and another fellow is pushing in the same direction simultaneously then very good chance that you will be imbalanced.

So, Xist does that. It will cause the compaction of the chromatin by recruiting histone deacetylase enzymes and on top of that it will make sure that once this acetyl group is gone that place it will be methylated also. So, that this compact just like putting the final nail in the coffin. It will make sure that it is compact forever. So, that work comes from the Xist.

So, Xist activity is regulated by another 40 000 nucleated long non-coding RNA that is on the opposite strand and that is called the two Tsix. Tsix and Xist are the opposite words. See Tsix you read from the right to left that is Xist Xist. So, why it is called Tsix, because Xist X inactivation specific transcript complementary strand contains the Tsix, but it is much larger much longer and which contains the anti-sense sequence of Xist and therefore, it is able to regulate the Xist activity by base pairing to it.

In the previous class when you were studying about the RNA stability. We saw in weak cell the inactivation because of the exosome action is in old cell it is weak in young cell it is strong. If it is strong, it can happily degrade the anti-sense strand. So, that the sensor strands or sense RNA is viable in old cell the inactivation does not happen or the degradation does not happen and hence the gene expression is compromised. We saw that example of gene regulation.

Similarly, if Xist and Tsix when they are expressed simultaneously and equally then there is no way you can have inactivation. So, it is a question, whether or not this Xist is complemented by Tsix RNA or not. So, Xist activity is regulated by 40000 nucleated long Tsix which contains the anti-sense sequence of Xist and therefore, it is able to regulate the Xist activity by base pairing to it.

So, now you have another round of regulation. Of course, Xist once successful it can cause the condensation or the compaction of the X-chromosome leading to the Barr body production; however, there is a catch whether Xist will get a chance to act or not. So, this is what you should remember at that depends on whether Tsix is expressed or not.

So, it is a kind of yin yang relation. Who acts first? The guy who woke up first will get like there is a saying right the early bird gets all the worms if the bird did not get up early worms would have gone, they would have come out had their food and gone back to their den.

So, early birds will get all the worms. Same way who expressed first Xist expressed first Tsix expressed first, if Tsix has expressed first even when if Xist is expressing it will be compensated it will be blocked, but if Xist work the first it will get a chance to go and pay and it will start the inactivation. So, it is a question of time who wins the race

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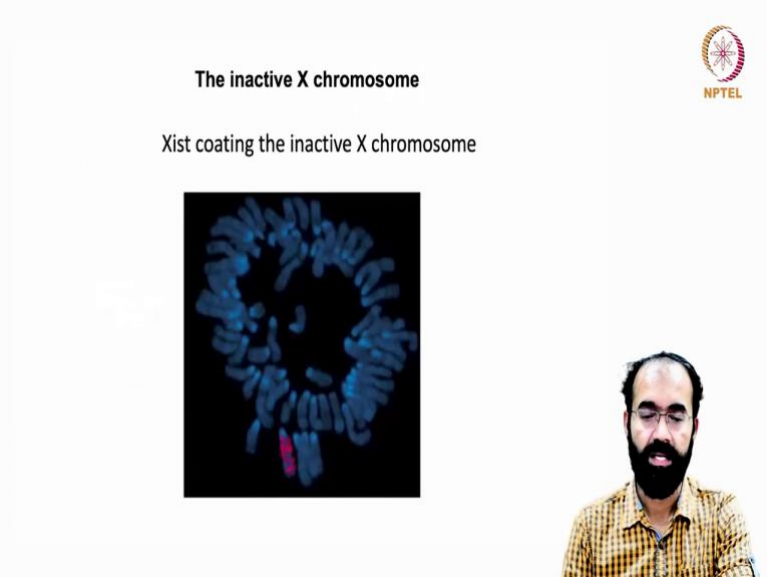

You can see a picture here that the inactive X-chromosome Xist coating happens on an inactive X-chromosome like this is a fluorescence in situ hybridization and this is taken under the microscope a picture. You can see the Xist bearing chromosome can be easily seen, because none of the other chromosomes have this. So, Xist is coated on to one of the X-chromosome not the other if two of them have then both have both are having Xist, but no only one will express Xist.

The other naturally will express the Tsix that is why the Xist is no more able to have an action. So, that also you should keep in mind. The other one the other chromosome is active credit goes to the Tsix not to the Xist, because Xist if it has expressed first, it would have been inactive. Both cannot express Xist together if so happens both the chromosomes will be inactive that should not happen.

So, now you have a question who will decide whether Xist should express first or Tsix will express first or which chromosomes Xist will or which chromosome Tsix will express first of this 2 X-chromosome. That is why this research become exciting and enigmatic and still ongoing debate and ongoing debate not on the concept debate on how it is happening So, we will see about that more in detail in the subsequent classes.

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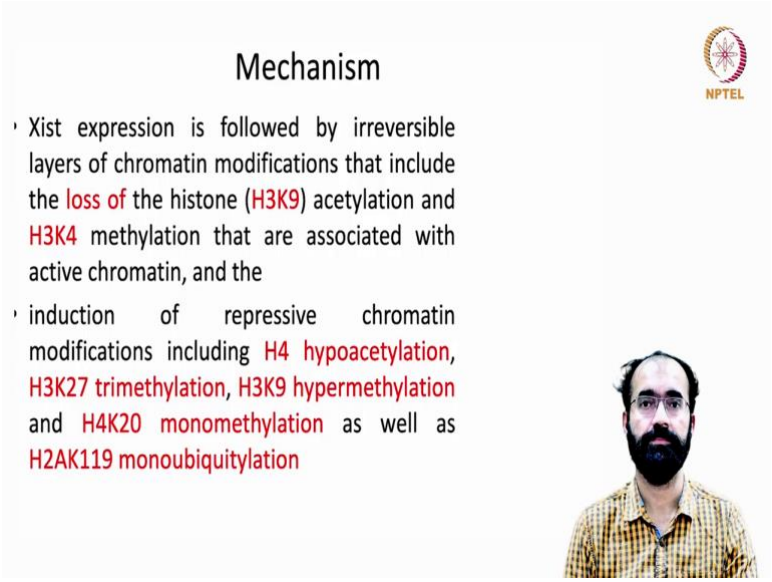

So, Xist expression is followed by irreversible layers of chromatin modifications that include the loss of histone that is H3K9. H3 stands for Histone 3. Different histone

proteins are there. So, we will not go into H1, H2, H3, H4 like that. We will not go into the detail, but one of the histone is H3 and K9, K9 stands for lysine 9.

Multiple lysine's are there, but K9. H3K9 acetylation is lost; that means, deacetylation happens and H3K4 that is histone 3 lysine 4 methylation is taking place and these are associated with active chromatin. So, what we see that Xist expression often followed by irreversible layers of chromatin modification that include the loss of H3K9 acetylation and H3K4 methylation. That are often associated with active chromatin and hence they are inhibited.

So, induction of repressive chromatin modifications including H4 hypoacetylation. You may have heard about hyper and hypo. So, if you say oh that person is hyper; that means, that person is overacting or overreacting or you may have heard about you know hypothyroidism or hyperthyroidism many people who are little lethargic do not feel like you know very lazy etcetera.

There is a possibility that there have they have low thyroid level and we often refer to them as hypothyroidism; that means, thyroid hormone or thyroxine hormone is low than required. So, that is hyper and hypo is a terminology we often use for referring to more than normal less than normal

So, H4 is histone 4 hypoacetylation; that means, acetylation is low than usual; that means, something related to the deacetylation and then comes H3K27 tri-methylation histone 3 lysine 27 tri-methylation; that means, more methylation groups are added onto it and then comes H3K9, hyper-methylation H3, histone 3, lysine 9, hyper-methylation can happen and H4K20 lysine 4 lysine 20 and histone 4 monomethylation.

That can happen as well as H2A a separate isoform H2AK119 mono-ubiquitylation that is addition of a ubiquitin moiety onto it. So, this is another change that can happen onto the histone. So, what we should understand that when you have this Xist coating has happened, it will decrease the loosening of chromatin.

That means, any modification that will loosen the chromatin it will decrease it and any chromatin modification that will accelerate the compaction that will make the chromatin dense or very tightly bound that will be accelerated just like I say a push and a pull.

So, if someone is pulling you and another person is pushing you from the same direction from the back then there is a good possibility like you may have seen on the road when people are loading some heavy equipment onto a truck, someone will be on the truck they will be pulling the item to the towards the truck some people will be pushing from the back. Same way the chromatin loosening events will be prevented chromatin tightening events will be accelerated.

So, these are some of the modifications which takes place during the compaction of the X-chromosome which is done as part of the dosage compensation. So, we will continue more in detail about the chromosome inactivation X-chromosome inactivation and the mechanism of dosage compensation in the next class.

Thank you.