RNA Biology
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Lecture - 42
Dosage Compensation and X-Inactivation: Imprinted vs Random X Inactivation

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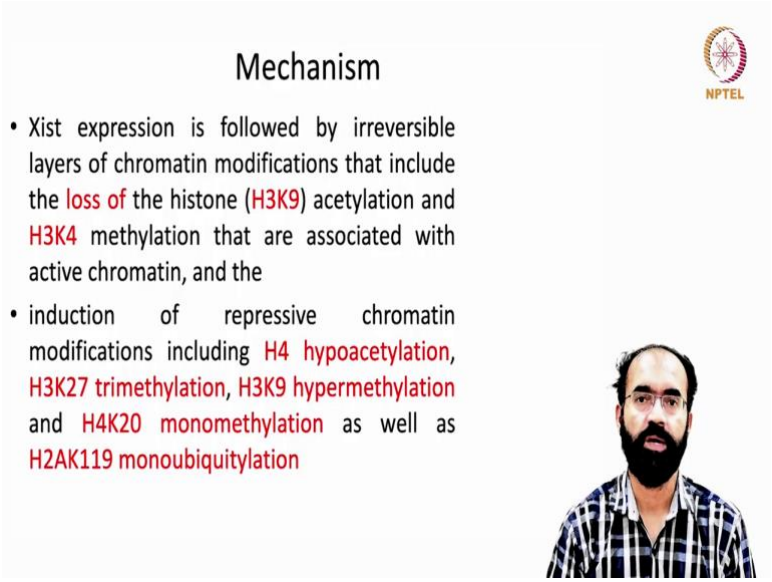

Hello everyone, welcome back to another session of RNA Biology. So, we were exploring the different mechanisms and the way in which X chromosome gets inactivated to maintain the dosage compensation. And in nutshell we know that the exist means X-inactivation specific transcript the non-coding RNA is accelerating the chromatin compaction or the DNA compaction.

So, it will facilitate those mechanisms that will prevent the loosening and loosening of the chromatin and accelerating the tightening of the chromatin. So, if you have acetylation chromatin becomes loosened, if you facilitate deacetylation chromatin becomes tightened and if you facilitate methylation that will become a tight chromatin. So, this is the main ways through which the exist is allowing the compaction or the condensation of the one of the two X chromosomes.

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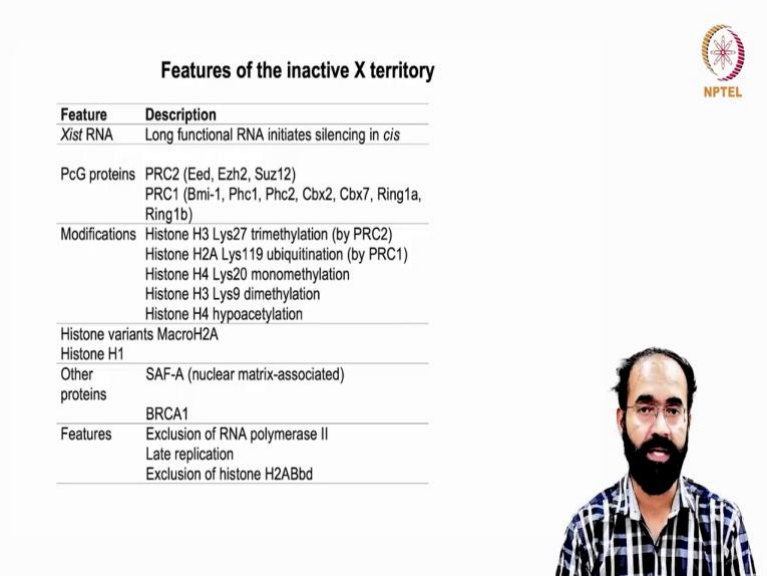

So, let us see what are the main features of inactive X chromosome or inactive X territory. First feature is Xist RNA itself. It's a long functional RNA that initiates the silencing in cis. So, cis and trans word we have used it. So, easiest way of understanding cis is within the same chromosome, within the same gene, within the same vicinity. If it is in a distance place that is called trans.

If you and your friend sitting in one room, you are talking to your friend while sitting in the room it is called cis. And if you are talking to someone over phone who is in another country or another state that is called trans in easier way of making you understand. So, Xist acts in cis that is on the same chromosome where it is being produced and it act on the same chromosome.

Then comes the Polycomb group proteins. They are called PcG proteins. What are they? They are mainly of two type PRC2 and PRC1. PRC2 is Polycomb repressor complex 2. PRC1 is polycomb repressor complex 1. So, PRC2 contains protein such as EeD, Ezh2, Suz12 etcetera. Many components are there and Ezh2 being the catalytic component catalytic molecule.

Same a PRC1 also have about plenty of molecules Bmi-1, Phc1, Phc2, Cbx2, Cbx7, Ring1a, Ring1b like that so on and so forth. So, these proteins congregate together onto the chromatin and it can facilitate specific modifications on the histone proteins because

of which you can create a repressive chromatin. That means chromatin that is not available for transcription that is the meaning of repression.

What are the modifications that takes place? Histone 3 lysine 27 trimethylation mainly done by PRC2 complex and histone 2A lysine 119 ubiquitination mainly done by PRC1 and histone 4 lysine 20 monomethylation, histone 3 lysine 9 dimethylation. Histone 4 hypoacetylation. So, these are all some of the related changes that takes place in the histone level. Histone is part of this chromatin.

So, without histone there is no chromatin and the modification chemical modification of histone decides whether or not that chromatin should be tight or loose. So, this is decided by the modification. If histone is tightly bound to the DNA, then it is a solid, tightly pack chromatin and we call it as heterochromatin. If it is loose it is loosely connected to the DNA. So, enzymes like DNA polymerase enzymes can come and act onto them effectively and we call them as a euchromatin.

So, histone variants macro H2A is specifically seen in the bar body or this inactive X chromosome and histone 1 is another feature that is seen associated with inactive X chromosome and there are other bunch of proteins such as SAF-A and that is a nuclear matrix associated protein and also BRCA1, BRCA1and that is also seen to be associated with this inactive X chromosome and also it exhibits some features some uniqueness. What are they exclusion of RNA polymerase 2?

We know what RNA polymerase 2 does to a DNA that will facilitate the expression of mRNAs. So, it is not welcome to this inactive X chromosome. If RNA polymerase 2 comes in there can be some expression that can sabotage the dosage compensation and then comes late replication. In a cell division we know in S phase normally cell have got multiple phase that is G1 phase, S phase, G2 phase and M phase.

M phase is for mitosis, G1 is the growth 1 phase and S phase is synthetic phase and G2 is the second growth phase and then goes into M phase. So, normally the DNA replication takes place in the S phase. If S phase all the DNA are replicated then it will get the green signal to move on to the G2 phase. However, the inactive X chromosome replicates very late just like a student coming late to the class.

So, he will miss many of the features, many of the qualities or many of the you know instructions given in the class. Like that this X chromosome will replicate very late and you can guess what could be the reason because it is so tightly compact it takes ages to unpack it cannot be done easily.

So, it takes lot of time to unpack it and this makes the replication very late and it comes with a price. The system cannot monitor whether is there any mutation, is there any trouble etcetera because there is no monitoring is happening. It is a discarded or neglected X chromosome and this is ok to some extent because it is not expressing.

But; however, if there is a chance by mistake if some leaky expression comes that can be a quite dangerous for that organism because that will accumulate lots of mutations usually because it is not been repaired because it replicates very late and once it is just to finish replication there is no quality control machinery can act on it.

So, this is what you should keep in mind and we will see some examples whether what is the implication of this later application has on to the mutation rates in the X chromosome. And exclusion of H2A Bbd. So, this is an isoform of histone 2A that will be excluded. So, these are all the three features associated with X chromosome.

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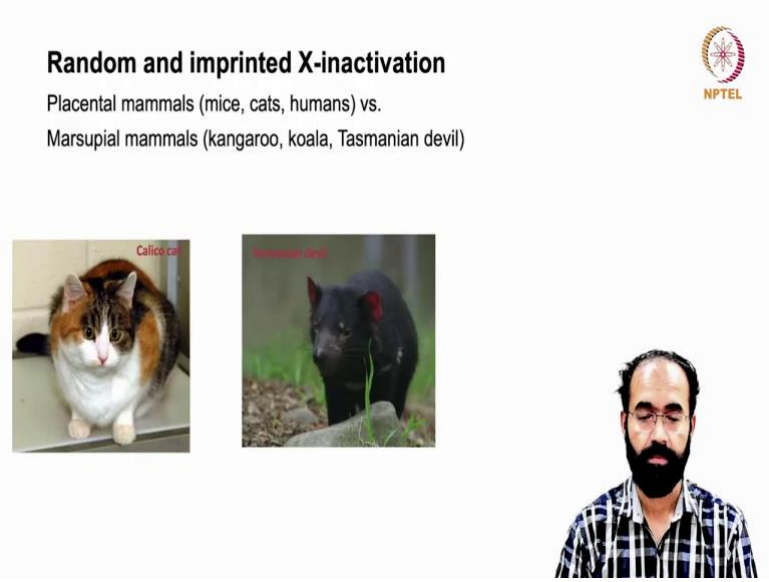

And we can see some examples of the imprinting. What is imprinting? Imprinting basically means something is been told to you straight forward. You are supposed to do

this like many such examples are there like if I give some examples of human behavior in imprinting it will be much much easy for you. What is imprinting? If you are supposed to behave so and so, like you know if you are going to school you are supposed to be your uniform.

If you are going to for a function you are supposed to wear decent dress. So, these are all something information which you get it from your parents. You will not go like a household dress to attend a function. So, certain behavior in human is imprinted for no serious reason. If you go to a function if you go to a big grand function just by wearing a shirt and pant nothing wrong with that. It's a civilized dress because it is civilized dress in the office. So, why not in a function? Why we call it as a party wear or a function wear etcetera.

Why such a different category should be there? It is imprinted in your mind. Oh, if I am going for and you may apply some perfume maybe you will put some you know makeup etcetera. Usually, you do not do that when you are going to your office or school or something you will go just like a routine person.

So, this is one example I can tell you imprinting. So, genes are also like that. Some genes they say ok this particular genes should not be expressed. Why? Because this is coming from paternal copy or father's copy or this gene should not be expressed because it is coming from maternal copy or this should be expressed. Why? Because it is coming from a father's copy. So, like this this indication or this instruction given is can be easily called as imprinting.

So, let us see how inactivation takes place in a imprinted. One imprinting we can say imprinting mechanism we can say in a female if two X chromosomes are there one must be made inactive. This is an imprinted behavior because it has to be its a rule cannot be violated.

So, you can see in placental mammals and marsupial mammals you know marsupial mammal means it will deliver the baby at a very small size and shape the development does not happen inside the womb very effectively and the small baby will grow inside a pouch mother's belly will have a pouch and it grow inside.

All of you have seen kangaroo's whenever you see a picture of a kangaroo naturally it will have a small baby. Of course, only for female such feature is there small baby inside the belly pouch of the mother. So, it keeps growing inside to a significant extent. So, they are the feature they have a marsupial pouch. So, that is why we call it as a marsupial mammal. Examples we know kangaroo, koala, Tasmanian devil.

So, these are some animals which follow a unique set of in X-inactivation which by default their paternal copy is inactivated. Whereas in placental mammal it is not like that like mouse, cat, humans etcetera. Initially it is the paternal copy that is inactivated later during the developmental stage there is a selection taking place selection in the sense a randomness is coming into picture. It can be either maternal or paternal copy can be made inactive.

Whereas marsupials they follow a steady state pattern of paternal X chromosome inactivation. So, it is an example of a cat and this cat is called calico cat and here you have an example of Tasmanian devil which is a marsupial carnivore that is the one only one member in the marsupial group which is a carnivore. Rest of the mainly herbivore or omnivore this is a strict carnivore means eating meat.

So, calico cat is something interesting like we will go into the details of why calico cat is looking like that we call a calico cat. So, if it has both yellow and black colour together. You may have seen cats with white colour and yellow colour or white colour and black colour or white alone or black alone or yellow alone. The moment you see a cat that has got black and yellow together white may or may not be there white is optional because white does not white is not a colour by the way.

Black is a colour yellow is a colour if you see a cat that has got black and yellow together do not need to look what gender it is because that must be a female. You do not have to bother no male cat will have both this colour yellow and black together. We will see why? All we can imagine is it has something to do with X chromosome.

Because X chromosome is randomly inactivated male does not have both the because females have all the chromosome in two pairs males also have all the chromosome in two pairs except a X chromosome where males have only one X females have got two X. So, that holds the clue and the coat colour is linked to the X chromosome.

So, I am not saying that one X chromosome always will have yellow or one X chromosome always will have black no not like that there can be both the chromosome black or both the chromosome yellow. So, that time you cannot distinguish, but if a cat has both this colour; that means, the X chromosome is randomly made inactive on the skin. So, we will see the genetics behind it.

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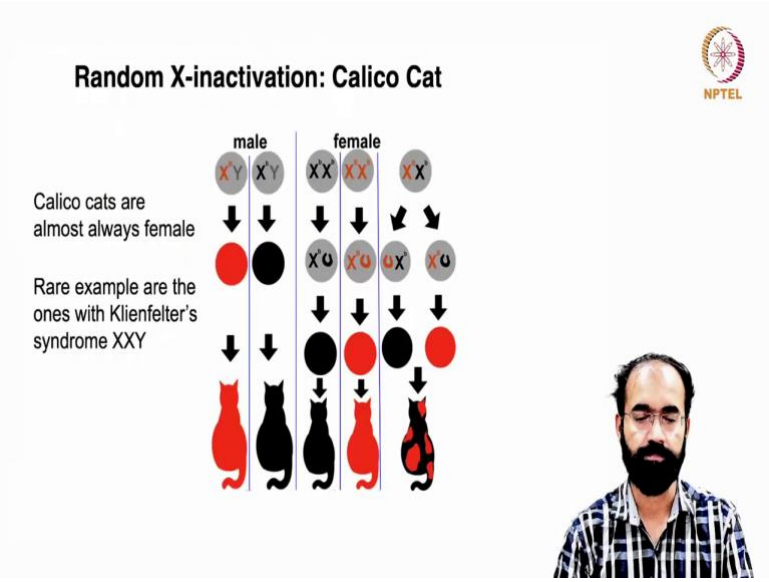

So, random XX inactivation in calico cat how does it work? In male it has got XY and XY. So, here you can see XY is written orange in colour and here XY one X is shown in black in colour Y is given common ash colour whereas, in female you can see two version. In fact, three versions one is two X black in colour, two X orange in colour and one X orange one X black.

We should understand the orange colour is dominant when it comes to skin colour orange colour is dominant over the black one in the case of calico cat. So, what we should understand if it is the in genetics you would have studied dominant and recessive you may have studied the Gregor-Mendel's experiment on *Pisum sativum*.

If you cross tall pea plant and dwarf pea plant in the f1 next generation all of them will be tall there is no intermediate size and there is no representation of the short at all, but in the next generation f2 if you self them you will end up getting 1 is to 3 is to 1 or depending upon whether it’s a mono hybrid, di hybrid accordingly you will have a variation.

So, what we should understand here X and Y is there we know that Y has no colour. So, naturally this animal will be orange in colour orange means yellowish orange. If male X and Y here the black one which is recessive not dominant.

So, here in the Mendel's experiment the tall colour is dominant short or dwarf one is recessive. So, dominant means it will show full-fledged one character whereas, short one has to have both the loci in a deployed organism it will have two locus both the locus it should have the characters governing the shortness then only it will exhibit.

So, we usually refer to this as homozygous recessive. So, think about this male if it has got orange that will be expressed and naturally this one can assume it is dominant in male if it has got one X that is having black colour the black will be expressed it is recessive, but there is no orange.

So, naturally it will be expressed in black colour and you can also assume no male can have both of them together because it is in the X chromosome how can a male will have both X chromosome together then that male has to be XXY that is not possible usually in female you have both the X chromosome which is meaning for black then the organism will look black in colour.

Why? It is called homozygous recessive it is having both the chromosome X chromosome that is black. So, it is a recessive character it will express let us think about both of them are orange and it is a dominant character both this animal look orange in colour. Then the problem comes when you have got X and X orange X and black X together.

So, if as per the Mendel's theory this organism should appear orange in colour because orange is dominant colour which should be the case, but that does not happen. Why? Because it is randomly inactivated it is resituated on a X chromosome. So, dominant or recessive comes into picture if you have both together it is just like you can play badminton and win a match if you have an opponent.

And you are playing alone means some people play chess both the movements you are only doing if you are doing both you win also you lose you lose also you win because both cannot win together both cannot lose together in the chess match.

Here what happens dominant and recessive comes into picture if both are there in one cell that does not happen. Why? It is in the X chromosome here what happens some group of cells will have black colour some group of cells will have yellow in colour that is what you see in this cat you have orange colour or black colour together.

So, it will be an intermediate size and that has to be a female and that female should have got this does not mean that this is not a female this is not a female this is also a female this is also a female, but it has got both the X chromosome having one colour although random inactivation is happening there also, but it is not detectable you cannot detect it.

But white can come there can happen in some group where this coat colour is not expressed at all then it will appear white in colour. Just like you know you may have seen in some humans also we call it as epistasis or albinism etcetera some people will have their brown skin, but some part of their body that will be milk white absolutely white because they are not producing melanin.

So, if you know if you do not produce melanin you will look like an albino and some people body some group of cells do not produce melanin. So, that has nothing to do with white is not a colour absence of colour is white.

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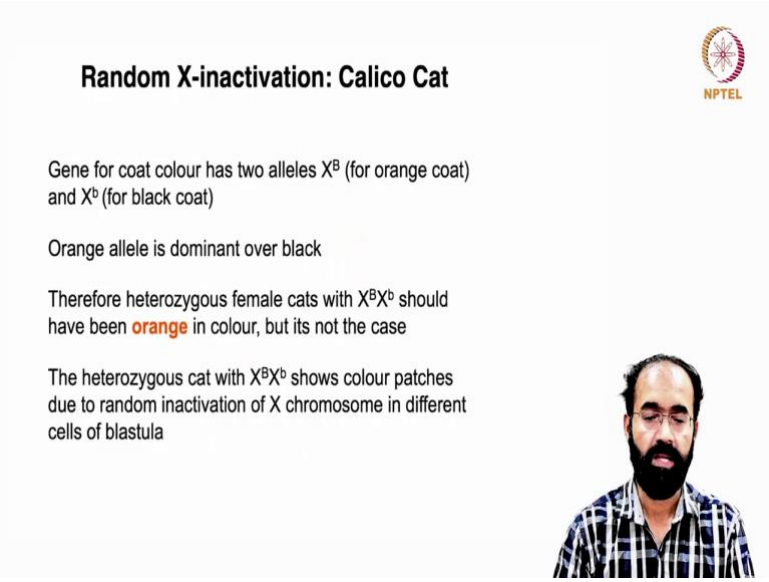

So, random X-inactivation in calico cat what happens? Gene for coat colour has got two alleles X b that is X the capital B indicates that is for orange colour coat. And then X

small b meant for black colour coat orange allele is dominant over black any given situation. Therefore, the heterozygous female cats with X capital B X small b should have been orange in colour technically, but it is not the case.

Why? The heterozygous cat with X capital B X small b shows colour patches due to random inactivation of X chromosome in different cells of blastula. So, why did not show orange? Because both X chromosomes are not expressing in one cell to have an orange colour. So, some cells the orange bearing X chromosome is inactive. So, naturally in those cells the other X chromosome is active. So, that will show naturally the back black colour. So, that is how the calico cat is found.

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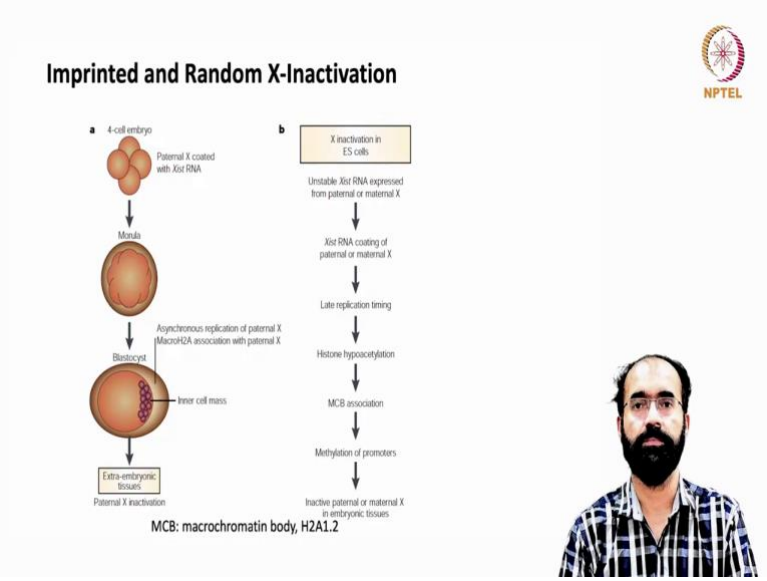

Now, let us see how the imprinted inactivation and random X inactivation. So we have like imprinting I already told you that imprinting is following a particular pattern with no obvious reason it is following the same pattern as it is it has to follow like in you may also see in some family grandfather was MBA and father is MBA son also MBA you will also see doctors.

So, grandfather is or grandmother is doctor son or daughter is doctor and their next generation also doctor advocate. So, this is I would not call it as imprinting, but that is what the message the instructions they get right from childhood. So, they also will have an inclination to become so and so.

Same way some genes or some chromosomes get an imprinted behavior in terms of its expression. So, X inactivation can be either random or it is directed or paternal copy is inactivated means father's X chromosome during fertilization father also contribute X chromosome mother also contribute to the X chromosome to a female in any mammals you are discussing.

But some animals one of them paternal copy is inactive in some of them anyone of them paternal or maternal. So, that is what let us see how does this happen. So, in a four cell stage embryo the paternal X coated with Xist RNA. So, you know if a chromosome X chromosome is coated with Xist RNA outcome is it will be made inactive.

And it will go until morula stage. Morula stage means a group of cells it has not informed the blastula structure I have to touch upon some of this embryology terminology you I am guessing that you have some idea about the embryology.

Because normally zygote is the single cell stage then it become 2-cell, 4-cell, 8-cell, 16-cell like that it goes up to around 500 to 1000 cell stage we call it as morula and after that it will become a blastula stage until blastula stage the cells have got stem cell characteristics means they can be called as embryonic stem cells.

After that they will start differentiating that is gastrula where the cells start becoming 3D that is three-dimensional; that means, you have ectoderm endoderm and mesoderm forming and the organogenesis and the organs are started forming and the embryo started developing.

So, until around morula stage, it is continuing as the paternal copy remains inactive. At blastocyst stage that is a stage in the blastula phase asynchronous replication of paternal X chromosome takes place and the macro H2A which is the chromatin or the histone associated with inactive X chromosome association with the paternal X is scene until the blastocyst stage and it will continue further for some more time and the extra embryonic tissue extra embryonic tissues are what normally amnion, allantois, chorion.

So, these are the main extra embryonic tissue and in some organism, it will be kind of replaced with you know yolk sac or some other associated structures a placenta etcetera. So, we will those who study embryology can understand this, but extra embryonic tissues can have a unique set of the embryo itself is giving rise to the extra embryonic tissue also

you should understand the fertilization takes place between sperm and the egg both are single cell stage.

So, if an embryo has to develop the entire thing has to come from the embryo itself. So, the extra embryonic tissue also derived from the zygote itself and this eventually becomes it will continue in the extra embryonic tissue also the paternal X inactivation stage right from four cell stage embryo onwards morula and blastocyst stage until then the X chromosome of paternal.

Means father's copy remains inactive and even this continues in the extra embryonic tissues like in case of human the prominent extra embryonic tissue is the placenta that is the attaching of the embryo to the uterus wall of mother through the umbilical cord. So, that uterus wall structure is called placenta and that is very important structure for the growth of the embryo. So, embryo and placenta or embryo and the mother is connected via the umbilical cord.

So, this umbilical cord derives nutrition from the placenta. So, placenta and other extra embryonic structures will have paternal X chromosome remains inactive. So, let us see what happens in embryonic stem cells. So, X-inactivation in ES cells unstable exists RNA expressed from paternal or maternal. So, the zygote the blastula stage I told you that resembles the embryonic stem cell.

So, Xist RNA coating of the paternal or maternal X is possible means some difference like in the normal embryo we saw it is exclusively the paternal copy whereas, in stem cell it can be either paternal or maternal and late replication timing happens with the Xist coated X chromosome and histone hypoacetylation takes place and the MCB association that is macro H2A chromatin association.

And a methylation of various promoters or on the genes which is present in the X chromosome takes place and inactive paternal or maternal X in the embryonic tissues takes place means in embryonic stem cell it can inactivate either paternal or maternal X chromosome in a uniform manner, but whereas, in an organism it is constantly maintained up to the extra embryonic tissue the paternal copy is made inactive.

So, this will of course, will change like later in the blastocyst there will be a randomization even takes place where either maternal or paternal can be made inactive in

the organism that is why like you saw in the case of cat randomly some Xist inactive randomly some Xist inactive. So, this will continue in the throughout the lifespan of the organism. So, we will continue more about the X inactivation in the next class.

Thank you.