RNA Biology
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Lecture - 48
Dosage Compensation, Xist and ncRNA in Imprinting: Genomic Imprinting in Action

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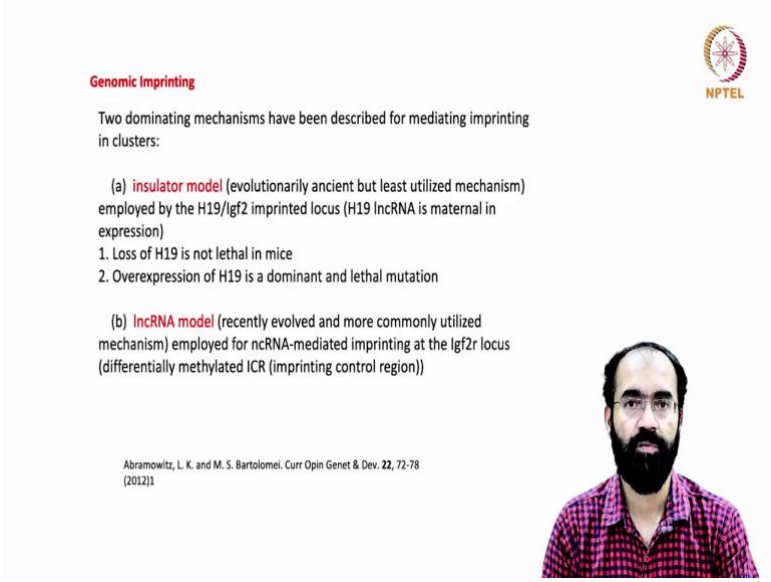

Hello everyone, welcome back to another session of RNA Biology. And we were discussing different models of genomic imprinting and the two dominating mechanism that has been proposed is one is insulator model and this is one of ancient mechanism of utilization of the genomic imprinting in a insulator model mechanism where the well studied example include the H19 Igf2 imprinted locus where the H19 lncRNA is regulating the Igf2 expression.

So, what we know from this H19 Igf2 imprinting locus is that the loss of H19 is not lethal means, absence of this negative regulation of H19 gene is not going to harm the organism whereas, over expression of H19; that means, you are inhibiting the Igf2 locus means over expression of H19 can be lethal in the mice.

So, the other model apart from the insulator model the lnc model is a recently evolved evolutionarily recently evolved and a well common and established utilization of lncRNA to repress the gene expressions. So, it is a non-coding RNA mediated imprinting

at the Igf2r locus and this causes a differentially methylated ICR Imprint Control Region. So, let us move further towards the imprinting.

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So, how the long non coding RNAs are contributing to the development? So, this Review Article is quite informative. So, you can read epigenetic mechanism of genomic imprinting, it is a well described quite loaded with information and so, this review will be quite useful because it also covers a lot of model organisms mammals, plants, insects etcetera. So, it is a quite informative Review Article. So, that is why I am putting the reference here, it would be quite useful to go through them.

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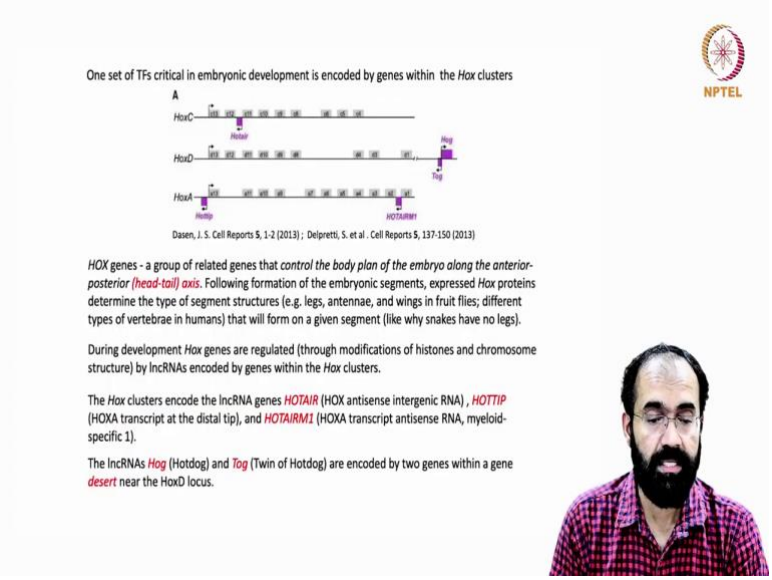

So, now let us see the one set of transcription factors that are critical in the development and it is included for the mainly it is required and necessitated for the embryonic development and this bunch of non-coding RNAs and we usually refer to them as the Hox clusters. So, Hox genes are in general they are homeobox genes, ok. So, homeobox containing protein groups, but they are regulated via epigenetic mechanisms and few of them are listed here HoxC, HoxD, HoxA.

So, this particular image is taken from an article published in Cell Reports few years ago and they are like you can see quite lot of this pink color naming that HOTAIR, tog, hog, HOTTIP, HOTAIRM1 like that. These are all non-coding RNA that are capable of regulating the genes in the Hox gene clusters.

So, what are Hox genes? Hox genes are a group of related genes that control the body plan of the embryo along the anterior posterior axis. Anterior posterior means head to tail axis. So, what is in a human body, what is the anterior side? Your head region is the anterior side. What is the posterior region? Your base where your waist region, your buttocks region is your posterior end.

Legs are of course, posterior, but that is your body stance is basically from head to base your waist region. Legs are attached to the waist and that is extending downwards. So, they are not always think about a four legged animal. Four-legged animal, two hands, two legs and the head is the anterior and the body base is the posterior end. So, your

body's posterior most end technically or embryonical part point of view it is the buttocks region and you are talking about the plan from your head till that base.

So, the legs are part of your pelvic girdle. So, or the projections that is coming from the pelvic girdle. So, your hands are attached to the pectoral girdle. So, anatomically speaking these two girdles define your body's trunk. So, head to tail axis is mainly regulated via the Hox gene clusters. Following the formation of embryonic segments, the Hox proteins are expressed to determine the type of segment structures like you know our body every vertebrate's body are segmented.

So, we do not see the segmentation; obviously, as you see in the case of a lizard or a worm or a snake or something like that you do not see or you can see in insects if you see a caterpillar worm you can see the body segmentation pattern even in vertebrates including humans you see the segmentation. Like body builders they always say you they have a six-pack belly six pack abdomen or eight pack abdomen they say.

So, these so, called packs are nothing but the segments in your body that are projected because of muscles. If your muscles are strengthened then you can see them in segments. So, segments are important for the easy movement of the body and also to provide unique musculature. So, every organism starting from fish to human their body plan is in segments and in embryonic stage they are very obvious.

But we should understand here in the Hox gene clusters point of view each segment have got a related structure externally as well as internally. Like in particular segment region you are supposed to have a particular bony structure a particular organ structure like I told you the pelvic girdle has got legs the pectoral girdle has got the hands. So, you do not want hands coming out of your belly right. So, it has to be in the same place. So, this is regulated by the Hox gene.

So, Hox genes make sure that each and every body part in your body including the vertebrae number in your vertebral column your vertebral column is made of 33 pieces. So, each piece you do not want one piece extra you do not want one piece missing. So, everything is regulated precisely by your Hox gene clusters during the development. Starting from insects onwards the Hox gene clusters are present.

So, the segment structures include such as the legs antennae wings in fruit flies fruit fly means the drosophila melanogaster and different types of vertebrae in human like you have a cervical vertebrae, you have got you know pectoral thoracic vertebrae, you know abdominal region vertebrae, lumbar vertebrae and towards the end like in other vertebrates which has got a tail the vertebrae the bony structure in your vertebral column will continue until the end of the tail.

So, during the development and also, we should understand in insects it is the wings, legs like arthropods have got three pairs of legs total six legs are there and in spider it has got four pairs of leg. So, each leg is attached to a segment. So, this is decided by the Hox gene clusters. So, also you can see in different vertebrae in humans and each of the segments will have a organ associated with that.

So, you do not want kidney in your chest region or lungs in your belly region right lungs should be in the place where lungs are there and kidney should be in the place where kidney should be there same with liver, stomach etcetera. So, during the development the Hox genes are regulated through modifications of histones and chromosome structures by lncRNA long non-coding RNA encoded by genes within the Hox gene clusters.

You can see the Hox genes plenty located here and HoxC you have got multiple exons HoxD multiple exons HoxA multiple exons and this pink color non-coding RNAs also located in the same region ok. So, we can see the Hox gene clusters also contain long non-coding RNA that are embedded.

So, the Hox gene clusters encode the lncRNA HOTAIR. What does it mean? Hox antisense intergenic RNA that is HOTAIR it is an acronym of this long sentence and HOTTIP what is that? HoxA transcript at the distal tip. What is HOTAIRM1? HoxA transcript antisense RNA myeloid-specific 1.

So, some of this non-coding RNAs are given name based on the location where they are present and some of them are given names based on condition in which a disease condition in which they are discovered and accordingly they are been given a name to start with and later on people find out that it is necessary for the normal development of this organism.

So, the lncRNA Hog that is a short form of Hotdog and Tog twin of Hotdog are encoded by two genes within a gene desert near the HoxD locus. So, there are a bunch of non coding RNA that are located in the Hox gene clusters that will influence the expression pattern of this Hox genes.

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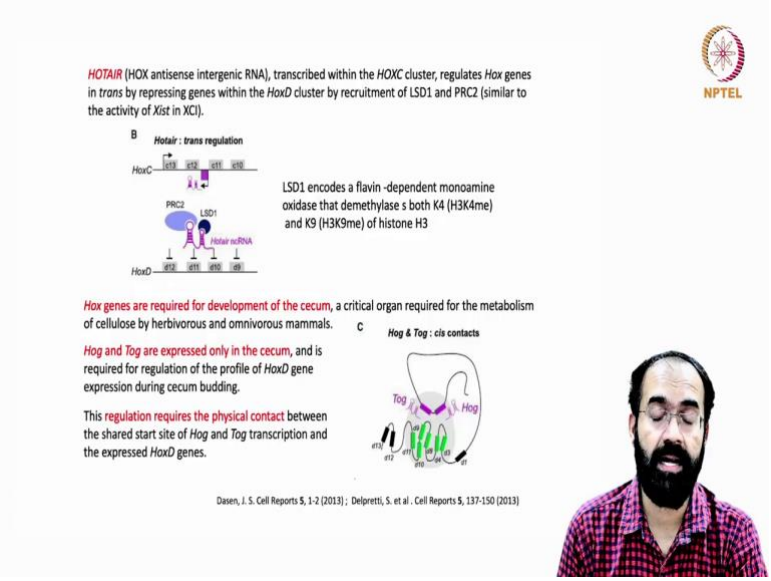

Let us see the role of hot air, hot air the name says Hox antisense intergenic RNA. So, it is transcribed within HoxC cluster it regulates Hox genes in trans by express repressing within the HoxD cluster by recruitment of LSD1 and PRC2 and it is similar to action that is done by Xist on X chromosome inactivation center.

So, what we should understand the HOTAIR while staying along with the Hox intergenic region it can influence the HoxC gene and it negatively regulates the HoxC gene expression or in other words negatively regulate does not mean that if it is if it is always turning it off. So, it makes sure that when it is transcribed in the HoxC cluster it can repress the HoxD.

So, when HoxC has to be expressed in that place if HoxD is expressed then that will give a leakiness of like segment 1 let us assume segment 1 you want HoxC and segment 1 HoxD also have a potential to express then segment 1 will have both HoxC as well as HoxD. So, this will give a confused identity to the segment. So, you have to make sure that the HoxC expressing segment should not have HoxD.

So, the hot air make sure that the HoxD gene should not be expressed in the segment where HoxC is supposed to be expressing. So, this is mainly done by recruitment of molecules such as PRC2 complex and LSD1 for causing a genomic imprinting means no expression or the methylation of the histones. So, that the HoxD cluster is not allowed to express.

So, and we call it this hot air regulation as trans regulation because it is present in the HoxC cluster, but it is expressing and going and influencing the transcribability of the HoxD cluster while recruiting proteins required for the epigenetic modification making the chromatic heterochromatic. So, the moment the hot air is expressed it will go and locate onto the HoxD locus and prevent its expression.

So, LSD1 encodes a flavin dependent monoamine oxidase that demethylase both HK4 and we usually refer to as H3K4 methyl group and K9 lysine 9, H3K9 methyl group of histone 3. So, it is LSD1 is mainly causing the demethylation function. So, Hox genes are required for the development of cecum. Cecum is a structure that is in the part of your digestive system a critical organ required for the metabolism of cellulose by herbivores and omnivores mammals.

So, cecum is considered to be an organ responsible for the digestion of cellulose. Cellulose is not an easy molecule to digest. So, Hox genes are one of the organs that is made because of Hox genes influence is the cecum. So, other two non-coding RNAs they are Hog and Tog and they are expressed only in the cecum and is required for the regulation of the profile of the HoxD gene expression during the cecum budding.

So, some non-coding RNAs have to make sure that that HoxD gene has to be allowed or it should be allowed to express in place where the cecum organ has to form. So, HoxD has got a connection with the cecum organ to form. So, Hog and Tog are expressed only in the cecum and is required for the regulation of the profile of HoxD gene and that is required for the cecum budding means it forms like a small bud during embryonic development.

So, we should understand that Hog and Tog can influence critically control the expression of the HoxD. So, this regulation requires the physical contact between the shared start site of Hog and Tog transcription and the expressed HoxD genes. They have somewhat common initiation of transcription.

So, they have common area like you can see here although they are physically located far away the expressed region, they come close to the HoxD gene and hence they can create a tight competition within terms of expression of the HoxD gene.

So, what we understand here that hot air make sure that HoxC gene is allowed to express not HoxD, but whereas, Hog and Tog make sure that the HoxD is expressed in place where it is supposed to be expressing. So, and in such places needless to say that other Hox genes also should not be there.

There are cases in sub-segments where two types of Hox genes are expressed because that will lead to a different. It just like if I want to give a simple example if you eat salt, it will give a taste. If you eat sugar, it will give a taste. What if you mix salt and sugar together? It will give neither or the both the tastes are there it will give you a unique new taste.

If you mix in water you will know, mix the sugar in water you will have a taste mix the salt in water you will have a taste mix the sugar and salt it together you will have a unique new taste. So, something like that even Hox genes also do in some segments where they express both the types of Hox genes.

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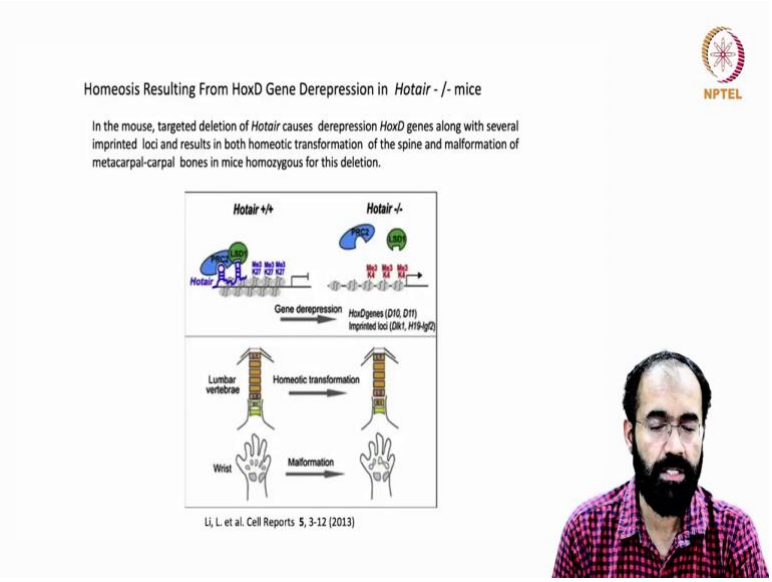

So, now we are hearing a new term homeosis. So, homeosis resulting from HoxD gene derepression in hot air knockout mice. So, we know hot air make sure that HoxD is

repressed not allowed to express in some region where hot air is present. Now, if you get rid of the hot air in a mice then naturally the repression even will not happen.

HoxD will not be repressed such a situation is called HoxD derepression. Derepression means absence of repression that is called derepression means expression is allowed. So, in the mice targeted deletion of hot air causes derepression of HoxD genes along with several imprinted loci and results both homeotic transformation of the spine and malformation of metacarpal to carpal bones in mice homologous for this deletion.

So, if you create a targeted deletion means you are not deleting from the entire animal you are deleting that is called conditional deletion. There are different tools you can use it for doing this I will not go into the detail, but time being you understand that this mechanism is called conditional deletion or in much a sophisticated manner I want to get rid of a gene only in your fingertip not in your liver or kidney or heart, I want to get rid of this gene only in your fingertips.

So, all I need to have is a loxp site flanking the gene and express a cre-recombinase in the fingertip. So, wherever cre-recombinase is there it will go to the nucleus and cut at these loxp sites and remove the gene and ligate back. So, that particular gene is removed only in your fingertip rest of the body it is there. So, this is called conditional gene knockout or conditional gene knockdown or targeted tissue specific gene knockdown something like that.

So, there are different ways I mentioned just a simplistic approach. So, if Hotair normally present what do you see that it will block the HoxD expression by recruiting PRC2 and LSD1 enzymes. And if Hotair is missing then PRC2 and LSD cannot do anything onto the HoxD cluster. Because of this what you see that you will have normal lumbar vertebrae; lumbar vertebrae is something near your belly region where your kidney, kidneys in the back of your belly region towards the vertebral column.

So, around your belly region that vertebrae I s called lumbar vertebrae. So, in homeotic transformation you will see some changes in the number. Let us count from here 1, 2, 3, 4, 5, 6 are there. Here 1, 2, 3, 4, 5, 6 are there. But interestingly these two look the same actually this plane 1 should have been 1, 2, 3, 4, but here the plane 1, 1, 2, 3 only what happened the fourth one is resembling like the bottom one.

Not only that in the wrist bone you are supposed to have 1, 2, 3, 4, 5, 6, 7. So, carpal and metacarpal bones that is present in the wrist. So, you see instead of 1, 2, 3, 4, 5 you are having only two, 1, 2 is fused, 3, 4, 5 is fused and 4 and 5 staying separately. So, instead of having 5 plus 2, 7 bone you are having only 4 bones that got fused. So, what we should understand that identity of a bone, bone is also a structure.

So, it has to be found in the place where it should be just like you think of such situation you have 5 fingers in your hand and they are in a particular place and what if thumb is in the middle of your hand or your yours my little finger is place of index finger. So, your little finger is in the place of your thumb. So, these are all called homeotic transformation and this change in the number, this change in the number of bones and we usually refer to as homeosis and homeotic transformation results in homeosis.

So, the credit goes to absence of a single non-coding RNA Hotair. Now, you can imagine if the Hotair was missing from the entire organism, this was a conditional deletion. It was deleted only in certain part of the body. So, this is from a published paper. So, we can read this research article if you want to know more about it.

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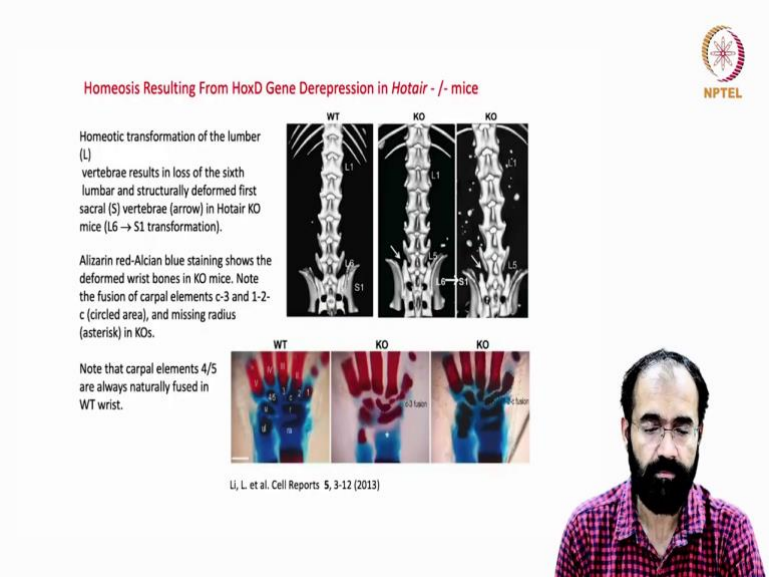

So, HoxD gene if it is derepressed because of the absence of Hotair, you can prove that this lumbar vertebrae is changed, this is wild type, this is knock out and this is another knock out. So, what you can see is that the L5, lumbar vertebrae 5 and 6, the 6 is seen in

the place of 5. So, lumbar vertebrae 5 should be a different vertebrae. Now, lumbar vertebrae 5 and lumbar vertebrae 6, both are looking like lumbar vertebrae 6.

So, this is a indication of the homeosis. So, homeotic transformation of the lumbar vertebrae results in the loss of the 6th lumbar and structurally deformed first sacral vertebrae that is shown marked with arrow and hot air knockout mice normally you see L6 to S1 transformation. So, L6 lumbar 6th vertebrae now is appearing like a first sacral vertebrae. So, this is another type of homeotic transformation.

So, if we can stain with alizarin red and alcian blue staining shows the deformed wrist bones in the knockout mice like you can see here they are been given number individual digits are given 1, 2, 3, 4, 5, 5th digit you have you can see them this is the wild type normally it should be the carpal and metacarpal bones are supposed to be separate.

But now you are seeing the fusion and many a time these fusions are random they are not predictable because it depends how effectively it is derepressing sometimes it can have leaky derepression also.

So, you can see the entire finger bone the number 1 finger it is fused with the carpal bone and metacarpal bones. So, you can see that the bone, bony structures are not discrete. So, this is one knock out this is another knock out. So, both are knock out. So, you see different types of changes different types of bony fusion.

So, here you can see the 6th lumbar vertebrae is now appearing like first sacral vertebrae. After lumbar vertebrae you have the sacral vertebrae coming which is towards the bottom of the vertebral column where this is your pelvic girdle region what you are seeing here where the legs are attached in mice you it will continue with the tail.

So, we will continue to see the importance of the homeosis and several non-coding RNA how the gene expressions are regulated how stringently the gene expressions are controlled in the next class.

Thank you.