RNA Biology
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Lecture - 51
Dosage Compensation, Xist and ncRNA in Imprinting: Xist and Cancer

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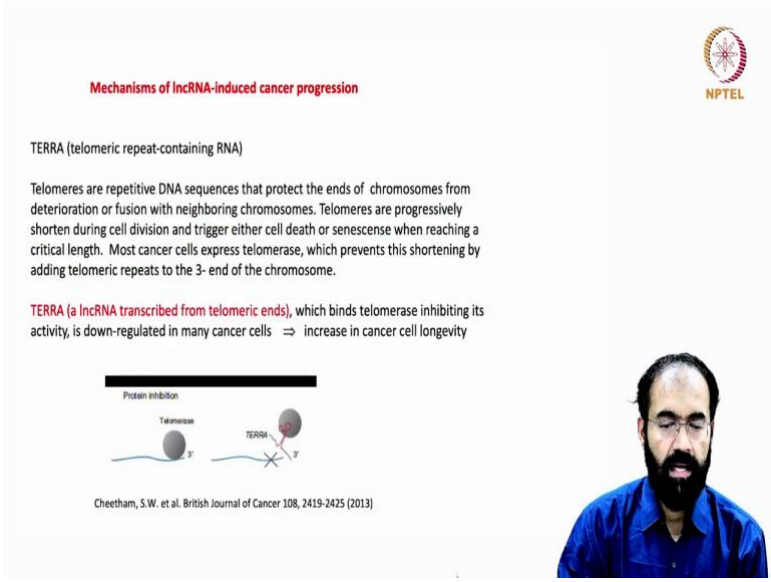

Hello, everyone. Welcome back to another session of RNA Biology. So, we were learning the roles played by non-coding RNAs in various gene expression events, developmental events and also diseases such as cancer, how they are contributing to various pathways, how they are contributing to tweaking of various proteins and their functions. And, eventually it helps in manifesting a given cellular physiology; sometimes it is beneficial, sometimes it is harmful such as cancerous condition.

So, we were here in the previous lecture that the non-coding RNA TERRA, the lncRNA transcribed from the telomeric ends, so, which can bind to the telomerase enzyme inhibiting its activity and it is down-regulated; this non-coding RNAs down-regulated in the cancerous cells, because of which the telomerase activity is high enabling the cancer cells to evade apoptosis or cellular senescence and subsequent programmed cell death.

So, cancer cells need to have that because they divide rapidly and there is a good chance that they will exhaust or reduce the length of telomeres much rapidly than a somatic cell. So, that make the cancer cell vulnerable to apoptosis, but because of this reactivated

telomerase activity; that means, the negative regulator of telomerase that is the TERRA is down-regulated in cancer cells and which will activate the telomerase enzyme, which makes the cancer cells more fit, more adapted and help them in evading the apoptosis.

So, what we should understand that the non-coding RNA helpful in certain biological mechanism will be manipulated in a cancerous condition and that will make the cancer cells eternal, they can leave forever.

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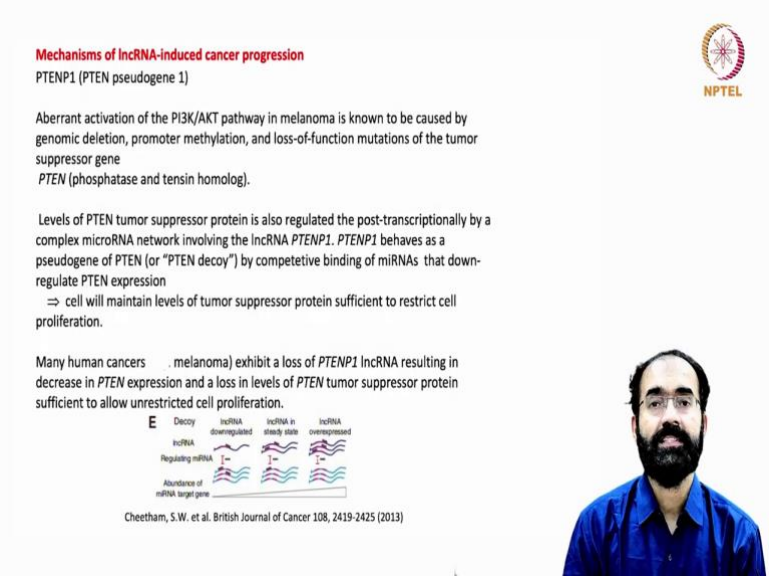

So, now let us see another example that is PTENP1. So, that is PTENP1 stands for PTEN pseudogene 1. So, the aberrant activation of PI3 AKT pathway in melanoma, melanoma is a type of skin cancer is not to be caused by a genomic deletion, promoter methylation and loss of function mutations of tumor suppressor genes.

We know tumor suppressors be kind of discussed about P53, what are the roles played by P53 in one of the earlier classes and there are several tumor suppressors are there in our body and one of the major tumor suppressor is PTEN phosphatase and tensin homolog. So, PTEN's role is to maintain the balance between PIP3 and PIP2, phosphatidylinositol phosphate, diphosphate and triphosphate.

So, it maintains the balance between these two. So, this basically a phosphatase and this balance is important to maintain a healthy level of AKT. So, PTEN when it is absent or the PTEN's activity is absent, the AKT level will go up and that can facilitate the cell

proliferation. So, AKT, mTOR pathway is a important pathway. So, PTEN as I told you is a dual specificity phosphatase, it has got phosphatase and tensin homolog as a full name.

So, the PTEN is a tumor suppressor also similar to P53, but the functioning is different not like P53. So, the levels of PTEN tumor suppressor protein is also regulated the post transcriptionally at the post transcriptional level by a complex microRNA network involving the long noncoding RNA PTENP1, it is name is PTEN pseudogene 1. PTENP1 behaves as a pseudogene of PTEN or we can also call it as a PTEN decoy.

So, pseudogene basically works in multiple ways. When you have a pseudogene for a gene; that means, when you call it pseudogene itself says like if you say a I phone mimic. So, when you say I phone mimic itself indicates there is something called I phone exist. You cannot say this is an XYZ phone mimic because there is no phone is there with a brand XYZ, right. So, when you say I phone mimic we should understand oh there is something called I phone exist which is having a defined function.

So, when you say PTEN pseudogene; that means PTENs function or PTENs value somehow the pseudogene can influence. Pseudogene can come from various reasons like sometimes pseudogenes will have some mutation. It mimics the actual gene, but it is not the actual gene. Sometimes pseudogene will have a lot in common to that of the actual gene, but it will not interact with the same counterparts or same proteins what it is supposed to interact as done by the original gene.

So, this we usually call the pseudogene as a decoy. Decoy means it can fool the system; that means, it will look like look like the original gene, but it is not the original gene. So, many times it will act like a scavenging molecule; that means, the molecules that interact with the PTEN will get competed out something like that. So, this is the PTENP1 does a competitive binding of the miRNA that is capable of down regulating the PTEN expression.

So, what will happen that the cell will maintain the levels of tumor suppressor protein sufficient to restrict cell proliferation. So, what we can understand is that PTENP1 behaves somewhat like PTEN, but it is not doing exact functions what a PTEN is capable of doing. So, even in the absence of PTEN cells are supposed to be proliferating because it is a negative regulator of it is a negative regulator of the AKT pathway.

So, if you are not having the PTEN functioning properly then what will happen the cell will proliferate? So, if PTENP1 is present what it will do it can it is protein it will this PTENP1 make sure that it will restrict the proliferation within the limits, but it cannot restrict as much as PTEN is capable of doing. So, in many human cancers such as melanoma they exhibit a loss of PTENP1; that means, in cancerous condition they have to divide actively, quite solidly, quite strongly.

So, what actually happen is that the PTENP1 when it is absent in melanoma that will lead to a very aggressive cell proliferation and very aggressive cancer. So, they exhibit a loss of PTENP1 long non-coding RNA resulting in a decrease in PTEN expression and a loss of a levels of PTEN tumor suppressor protein is sufficient to allow unrestricted cell proliferation.

So, what we should understand is that what we should understand is that the PTEN decoy that is the PTEN decoy is the PTENP1 is absent from it is absent from the melanoma cells that can lead to the much-prolonged decrease in the PTEN levels itself because PTENP1 is acting as a decoy and, the PTENP1 expression is missing.

And, this eventually leads to decreased levels of PTEN because what we should understand is their expression the PTENP1 and PTEN expressions are kind of monitored and they are linked. So, levels of one can influence the level of the other.

So, in melanoma what we say loss of PTENP1 long non-coding RNA results in a decrease in PTEN actual PTEN expression and a loss of levels of PTEN because which is a tumor suppressor. So, the absence of PTEN will cause an elevated level of AKT and this in turn will facilitate the proliferation. So, now let us see how does it work?

The long non-coding RNA is down-regulated and normally it should be in a steady state level and this the third situation is a over expressive situation the long non-coding RNA is over expressed. So, when you see the RNA levels when they are changing based on the cancerous condition the mRNA target gene also will get affected for the PTEN which is tumor suppressor.

So, the decoy and the actual gene will have a strong interrelationship which will be affected very badly in the case of melanoma cells, but it is influencing the PTEN levels through the PTENP1.

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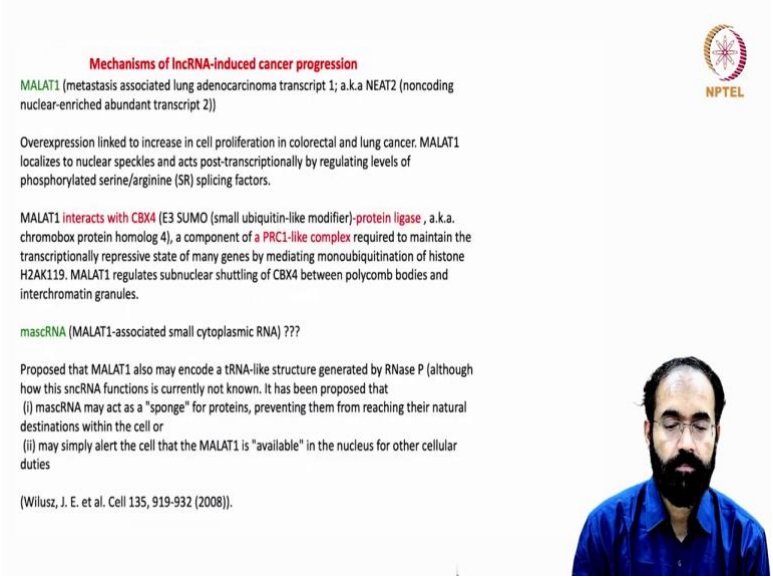

So, another example we can see about the long non-coding RNA leading to the cancer is a MALAT1. What is the full name of this non-coding RNA? Metastasis associated lung adenocarcinoma transcript 1 and this has this gene have got another name that is NEAT 2 that is non-coding nuclear enriched abundant transcript 2.

So, the overexpression linked to an increase in cell proliferation when MALAT1 is over expressed it can cause a increase in cell proliferation in colorectal and lung cancer. So, MALAT1 localizes to nuclear speckles and acts post-transcriptionally by regulating levels of phosphorylated serine arginine and it is in short form it is written as SR proteins splicing factors. So, SR splicing factors are affected in the over expressed condition of MALAT and in cancerous condition it is MALAT1 is over expressed.

So, MALAT1 interacts with a another protein called CBX4 and which is a E3 SUMO a small ubiquitin-like modifier that is called sumo protein ligase. So, CBX4 is a SUMO like protein ligase and it is also known as a chromobox protein homolog 4 which is a component of PRC1 like complex not actual PRC1 PRC1 like complex that is required to maintain the transcriptionally repressive state in many genes by mediating the mono ubiquitination of histone H2A lysine 119 H2AK119.

So, MALAT1 regulates the sub nuclear shuttling of CBX between the polycomb bodies and intrachromatin granules. So, MALAT acts as a mediator of the CBX. So, this can lead to a apparent cell proliferation control especially seen in cancer cells. So, another

non-coding RNA that comes into picture is masc RNA that is MALAT1 associated small cytoplasmic RNA. So, it is proposed that the MALAT1 also encode a transfer RNA-like structure generated by RNase P action which is a ribozyme.

Although how this sncRNA functions is not fully understood yet it is proposed probably that mascRNA may act as a sponge for proteins preventing them from reaching their structural distance. The structural means the interaction natural destination points and where it is supposed to be actually present within a cell or it can also act maybe simply via alerting the cells that the MALAT1 is available; that means, it can act as a mimic in the nucleus for the their nuclear duties.

So, many times the non-coding RNAs will act as a decoy for an mRNA. Sometimes this related non-coding RNAs can influence or act like a sponge for their related RNA structure. So, this will cause an imbalance; that means, it can fool the system. Absence just like if you see something there is English there is a saying also right that all the glitters are not gold.

So, what indicates? The glitters immediately give you a connection to that of gold. That is why if you see many a times the glitters will be you would not see usually a glitters in some odd color, usually they will be golden or silver color usually. You will not people do not use glitters in all possible colors because the golden color glitters will have a fascination in humans mind it connect into gold.

So, we should understand non-coding RNA also will fool the system saying that an absent RNA will be reported as present, but actually it is not present. So, the system will shut off the actual RNA which is in need, but it has been fooled. Like people say in some like in some places like some city's city walls I do not know which city like if the city is not having a proper beautiful structures etcetera, so, they will paint the walls with nice beautiful building structures.

So, the existing wall will appear like buildings so that you will have a rich feeling. Some shops also you see like clothes shop or even jewelry shops they will put mirrors. So, through the mirrors when you are reflecting and seeing too many objects then you will feel the shop is much bigger and much vast.

So, many a times this mimicking RNA also will do the same impact, the same effect to the system. Actually, it is a mimic. It can also sequester some of the proteins where the actual RNA is supposed to be pairing, but the levels of this mimic RNA make sure that the actual RNA level is also kept in check. So, such kind of mechanism do exist.

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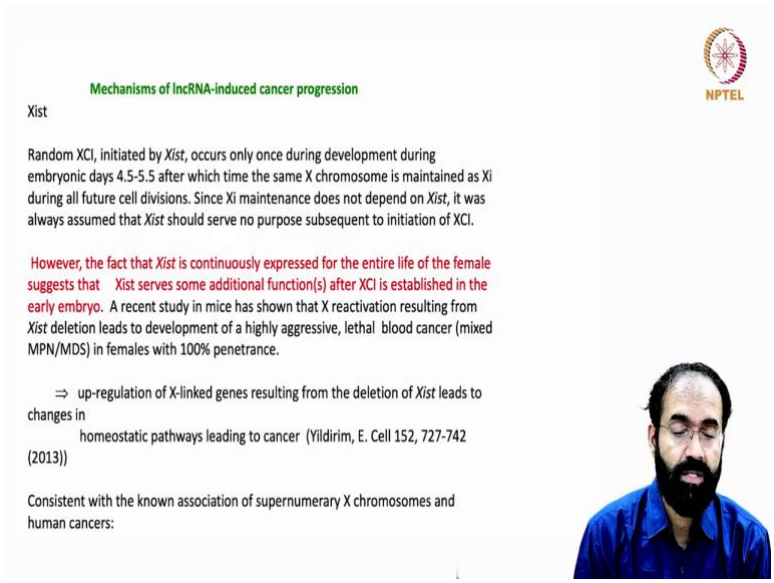

So, there is another non-coding RNA is Xist which we have studied properly that is helpful in the X chromosome inactivation and it is initiated by X chromosome inactivation specific transcript. And, it occurs only once during the embryonic development and at around 4.5 to 5.5 days of embryonic development.

And, this time the X chromosome is maintained as X inactive during the future cell divisions. Since X chromosome inactive maintenance does not depend on Xist, it was always assumed that Xist should serve no purpose subsequent to initiation of X chromosome inactivation.

However, what we know that the Xist is continuously expressed for the entire life of the female. This suggest that Xist serves as additional functions apart from what we know after the X chromosome inactivation is established the Xist continue to be expressed with a purpose. So, a recent study in mice has shown that the X reactivation resulting from the Xist deletion leads to development of a highly aggressive lethal blood cancer.

So, what we understand the Xist non coding RNA have other functions also. So, this is called mixed MPN MDS in this also creates a multi drug resistant type of cancer in females with 100 percent penetrance. The penetrance and expressivity is a genetic term probably you have already heard the penetrance basically sees whether or not you have the availability of a given gene.

Expressivity means what is the level of expression of that gene based on what you have got that also can vary. So, if it is just like you have a pen and paper, if you have 10 people and if you give pen and paper to them, they may or may not utilize it one thing that is penetrance. And, expressivity means some will write a letter and some will make a beautiful art form and some will just scratch on the paper and ruin it. So, this is expressivity.

So, this what you say here is that Xist here continuously expressed for the rest of the life of the female and we know from a study that the X reactivation can result from Xist deletion because reactivation is happening in the absence of Xist indicates that Xist was needed throughout the life and this leads to the development of. So, X if it is reactivated due to the absence of Xist it can lead to a highly aggressive cancer and which is 100 percent penetrance means no one is exception, everyone has the same phenotype.

So, the up-regulation of X-linked genes resulting from the deletion of Xist leads to changes because if the X chromosome is activated then gene expression also happens from this active X chromosome and they are important they have they cause this aberrant activation cause changes in the homeostatic pathways leading to cancer. Many a times many homeostasis pathway does adequate cell proliferation, adequate cell metabolism, adequate free radical production etcetera.

But, when you have more than adequate more than required then they will overdo it. It is just like you will chew your mouth if food is there. So, you are biting your both the jaws with the food in between, but if you constantly bite without food in between what can result your enamel will erode.

Eventually your teeth will erode. Your teeth is not supposed to grind against an equally strong material. You do not take stone in your mouth and grind it. If so, happens your teeth will be short lived. So, you cannot have a long-lived teeth.

Same logic applies even if they are housekeeping pathways if they are overly expressed, if it is overly doing that can leads to situation like cancer. So, this is consistent with the non association of supernumerary X chromosome and several human cancers. So, we should understand that the non-coding RNA no matter whether it is important for maintaining the homeostasis or it is a essential molecule that allows the cell to survive from a juncture or this non-coding RNA is a decoy for another non-coding RNA.

Irrespective of the actual role played by this non-coding RNA what actually happens is that it can influence the normal functioning of a cell sometimes it can lead to cancer and sometimes it can lead to aberrant apoptosis either extreme are possible. And, we have seen several examples of non coding RNA that is governing the homeostasis and sometimes leading to formation of tumor. So, we will move on to a new topic that is maintaining of the telomere and enzyme responsible for that is telomerase.

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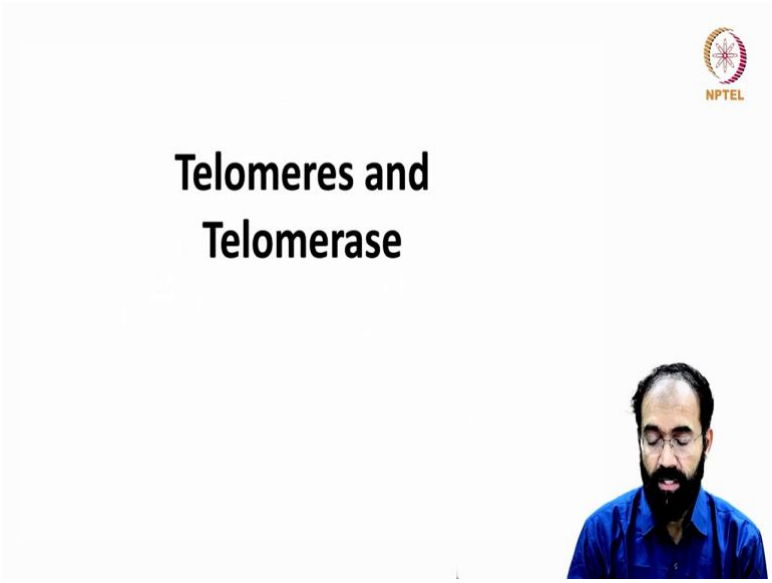

So, telomeres and telomerase are a enigmatic and yet well studied yet too much important for it is for the normal survival of a cell. So, we can say it is something like needed, but not needed. So, what we should understand the telomere is an essential feature, but it is a shortening also is an essential feature.

So, although shortening is it is just like you know human if someone says humans or any species death is essential. You may wonder oh what this person is saying, but actually you can imagine if each and every organism on this planet do not die, but there is no

scope for variety, no scope for new species, all the nutrition's and you know minerals everything will get trapped in some organism.

So, plants also has to die animals also has to die even bacteria also has to die. Every organism appear, reach its peak and disappear. So, this is important. So, technically death is a way of recycling the nutrients, minerals and various other materials you accumulated from this nature. So, that has to be released back. So, that is possible only through death. So, telomere and telomerase also something like that.

So, telomere maintenance is done by telomerase and telomerase is an important molecule, but if telomerase is present everywhere it becomes unimportant molecule or useless molecule or you can even say, it is a harmful molecule. The cells also have to die at some time point and that death is with a purpose for new cells to form. It is just like wildfire.

If someone ask, is it wildfire important and everybody will say no wildfire should not be there, but in nature point of view wildfire it may appear cruel, but once a wildfire is done there is scope for lot of new plants to grow. It will make the ash is lot of rich in nutrition. Same with a volcano eruption volcano eruption kills lots of living organism, but the nutrients it brings in makes the volcanic soil is rich in nutrient and the plants can grow nicely.

So, telomere and telomerase has to be seen not just like a scientific, way it also has to be seen in a philosophical way.

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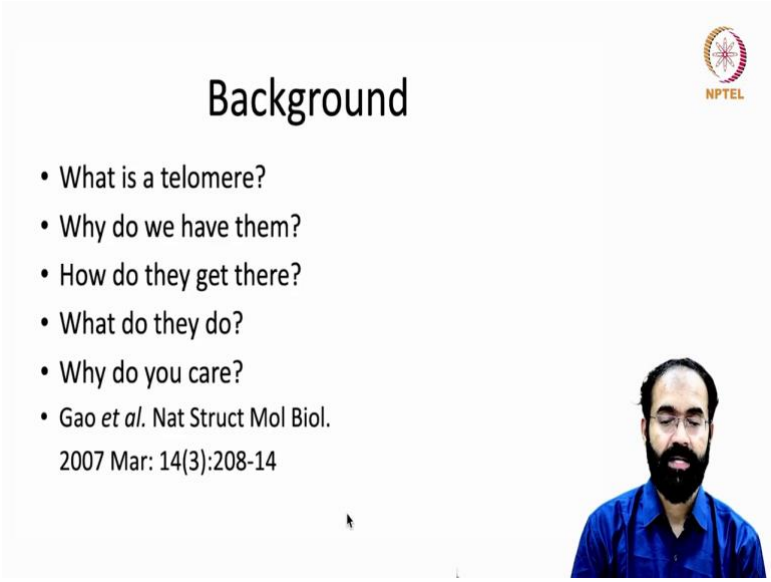

So, at the background we have to ask what is telomere and why do we have them and how do they get there and what do they do; why do you care about them and you can see a lot about this telomere in this nature structural and molecular biology article by Gao et al. So, it is a quite interesting molecule.

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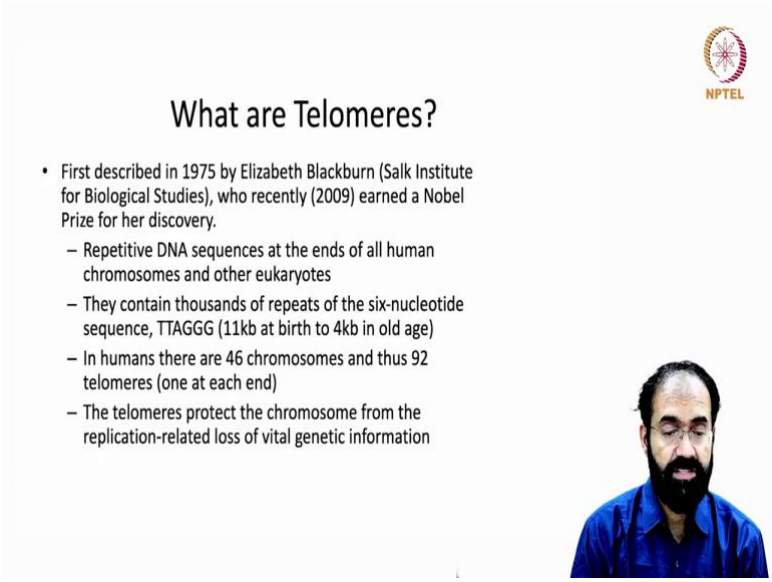

What is telomeres? Telomeres first described in 1975 by Elizabeth Blackburn at Salk Institute for Biological Studies, who recently in around 2009 earned Nobel Prize for her discovery because it is so important. So, their repetitive DNA sequences that ends all

chromosomes of other every eukaryotes who have got a linear DNA structure. Our chromosomes each chromosome is a single linear DNA structure, it has got two ends.

So, they contain thousands of repeats of six-nucleotide sequence T2AG 3 TTAGGG. It is 11 kb at birth to 4 kb in old age 11,000 bases are there at the time of your birth and as you grow old it becomes around 4 kb in length. In humans there are 46 chromosomes and 92 telomeres, 46 into 2, 1 at each end. So, the telomeres protect the chromosome from the replication related loss of vital genetic information. We will learn more about telomeres in the next class.

Thank you.