RNA Biology
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Lecture - 58
Epitranscriptome and Protein Synthesis: Important RNA Modifications

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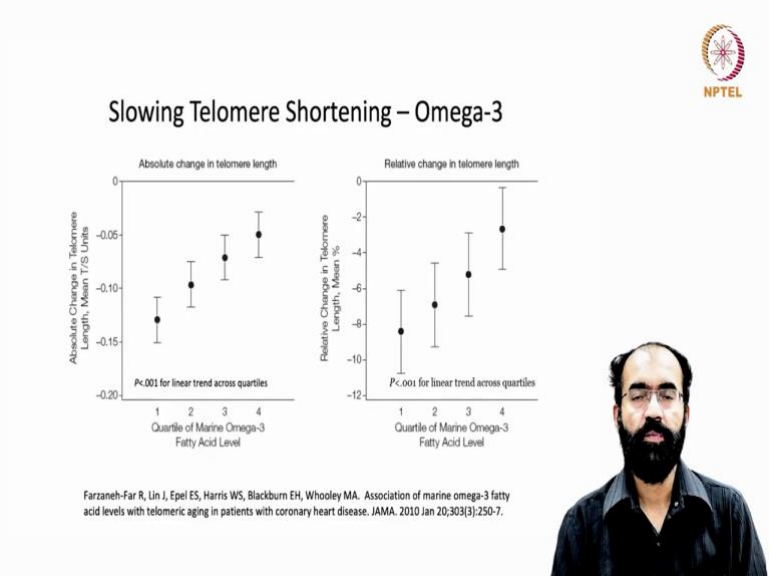

Hello everyone. Welcome back to another section of RNA Biology. So, we were here in the previous class, we were learning the importance of vitamins and omega 3 such kind of molecules in real time effecting affecting the length of telomeres.

And we believe that a lot of such molecules based on the data, based on the data in available from the populations and also in experimental models, we have shown that the telomere length remain quite healthy, if the people are taking a healthy diet and continue to be living in less stressful environment, and longer the telomere length, the less likely the cell will undergo apoptosis or cellular senescence. So, that is the take-home message.

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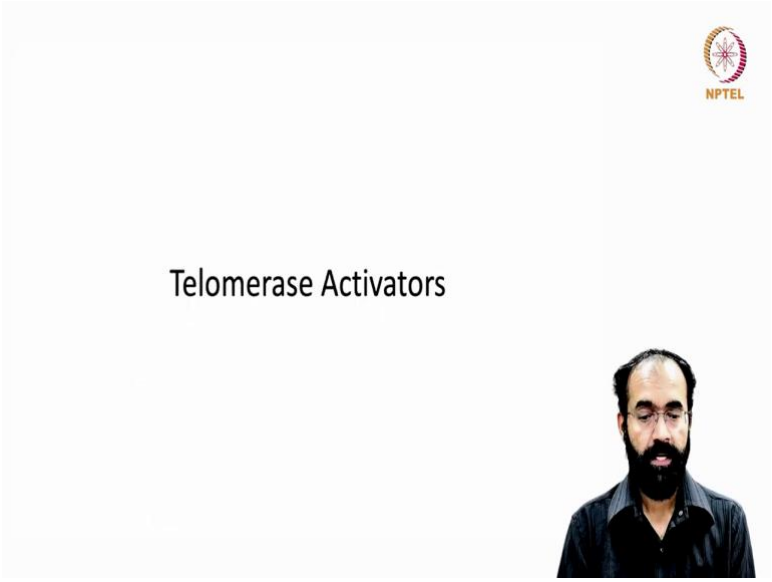

Now, let us think about some telomerase activators. So, telomerase is an enzyme which extends the length of telomeres. And we know, somatic cells do not have telomerase activity even if it is there very marginal. And we also saw some non-coding RNA regulating the telomerase function in the previous classes.

So, we should keep in mind that there is still scope for enhancing the telomerase activity, not to a level that it can cause cancer etcetera. So, do not assume that activating telomerase is leading to cancer. The answer is no. If that is the case stem cells and germ cells should not have telomerase activity.

So, telomerase activity do not cause cancer, but high telomerase activity is a necessity for the cancers to survive. So, telomerase activity do not cause cancer, rather it is a boom. But evolutionally our somatic cells do not have telomerase activity as it has in the stem cells as well as in the germ cells. So, can we think of activating the telomerase?

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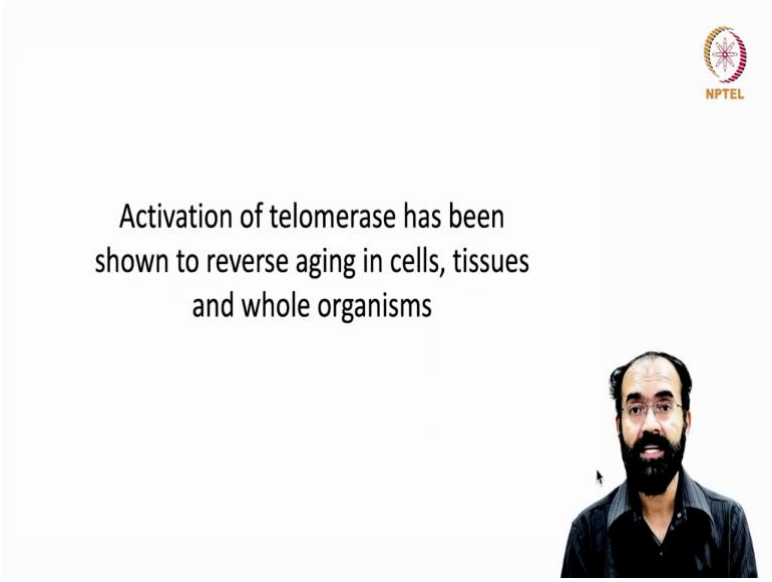

So, activation of telomerase has been shown to reverse the aging process in the cells, tissues and even in the whole organisms. So, let us think more about it.

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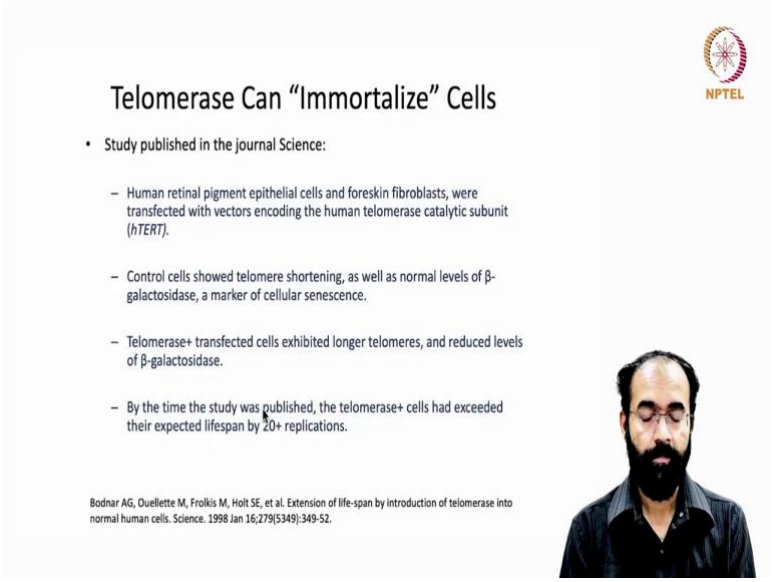

Telomerase can immortalize the cells and we have seen that one in around 10 million cells can gain this telomerase activity and that will lead its way into immortalization. So, we know that. So, a study that is published in the journal Science have shown that human retinal pigment epithelial cells and the foreskin fibroblasts, were transfected with vectors encoding human telomerase catalytic subunit hTERT.

And the control cells show the telomerase shortening as expected whereas, the marker, the marker of cellular aging that is the beta-galactosidase is found to be at normal levels in control cells.

However, when you express the telomerase; excuse me; the transfected cells exhibited longer telomeres and reduced the levels of beta-galactosidase. So, what it indicates, cellular senescence as reflected by the marker the beta-galactosidase itself is reflected. That means, the cell is not planning to undergo senescence because of the presence of telomerase.

By the time the study was published, the telomerase expressing cells had exceeded their expected lifespan which is more than 20 generations means what is the maximum number of generation that cell can live in a culture condition beyond that it went to 20 more generations. So, this is a interesting supportive data to show that telomerase or the activators of telomerase can reduce the aging of the cell.

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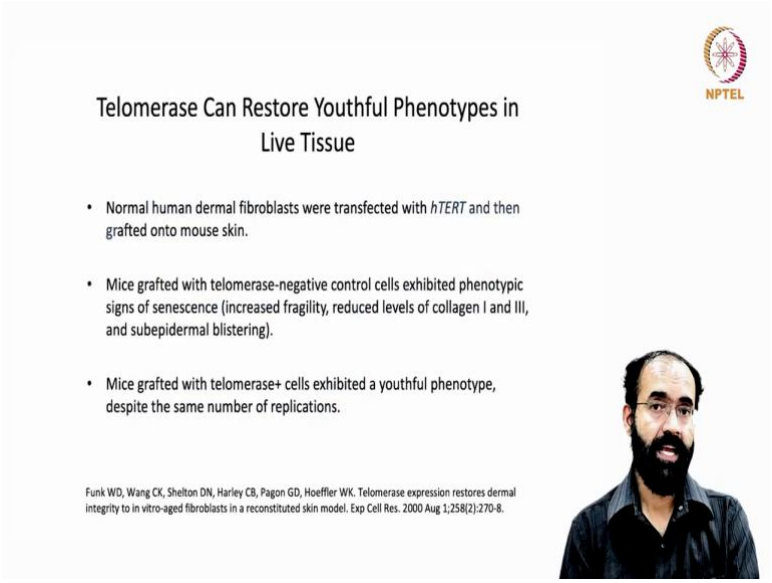

So, telomerase can restore the youthful phenotypes in live tissue. So, normal human-dermal fibroblasts were transfected with hTERT and then grafted onto mouse skin, on the skin of the mouse, and where you have reprogrammed this hTERT. Means over expression of the hTERT which is the telomerase catalytic component.

So, mice grafted with the telomerase negative control cells exhibited phenotypic signs of cellular senescence that is increased to fragility, reduced the levels of collagen 1 and 3, and sub epidermal blistering that is sign of old age. So, what happens? Mice grafted with the telomerase expressing that is hTERT expressing cells exhibited quite youthful phenotypes, very youthful phenotypes, and despite the same number of replications.

So, it is not that telomerase expressing cell is not dividing or something, it is happening, dividing everything as usual as a control cell. However, being a proper telomere length available in this hTERT expressing cell, they did not exhibit the senescence and cellular senescence. So, these data suggest and support the view that telomerase can be reactivated for an extended tissue life or cellular life.

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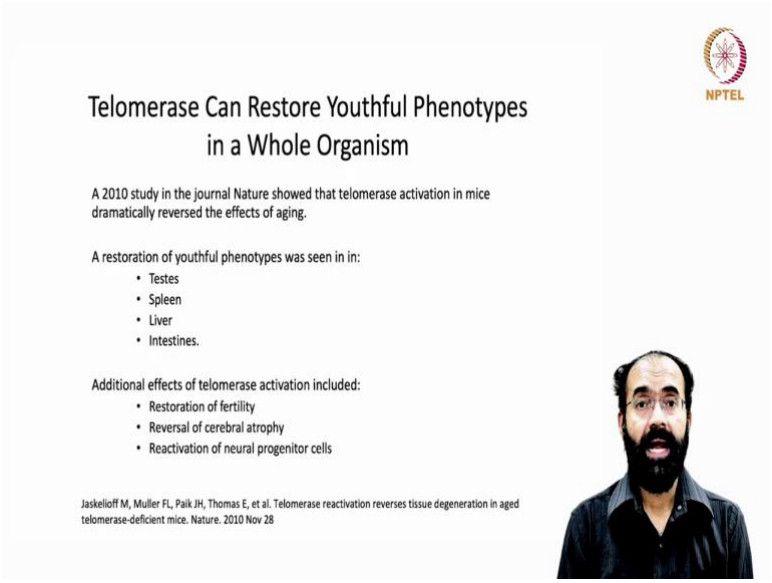

And the telomerase activation can restore the youthful phenotypes in the whole organisms itself. A study conducted in 2010 show that was published in the nature, the general nature it shown that the telomerase activation in mice dramatically reverse the effects related to aging. So, a restoration of youthful phenotype was seen in the organs such as testis, spleen, liver and intestine. These organs are used mainly because they have a accelerated rate of cell replication or cell division.

So, additional effects of telomerase activation included restoration of fertility, reversal of cerebral atrophy, reactivation of neural progenitor cells. So, these positive effects also contribute to keep the brain young or the performance of the brain quite active, as it

happened during the young age, and fertility because any way the germ cells have a telomerase activity. However, the starting cells of the organ testis also have the telomerase activity make them in a much sustained condition as it occurs in the young age.

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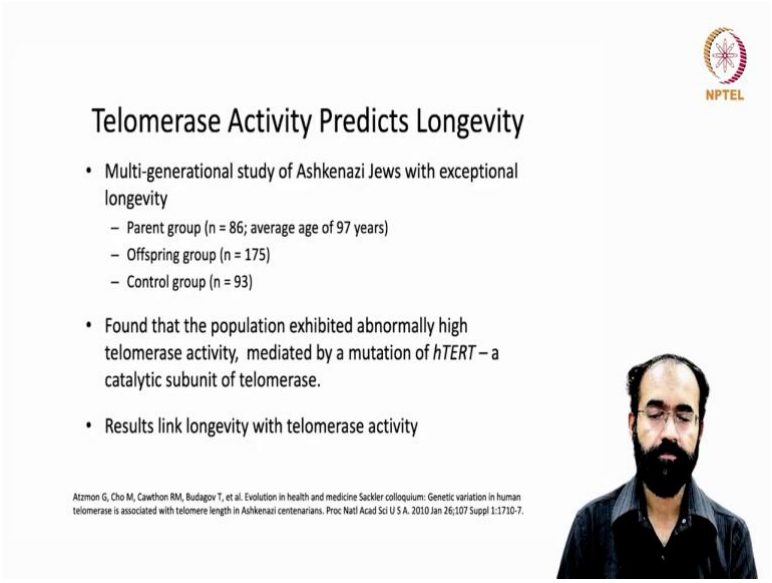

So, telomerase activity also can be an indicator of the longevity. So, several experimental studies have shown that multi-generational study by Ashkenazi Jews, it is a; it is a community of Jews. You can read a lot about Ashkenazi Jews, they also have also a lot of (Refer Time: 07:58) managers and (Refer Time: 08:00) related stories also you can hear a lot about Ashkenazi Jews. And they have been used a lot, used means they have been used extensively in genetic studies.

No humans can be used in a genetic study, but the data can be used for study. That has happened with Ashkenazi Jews a lot. So, they are been known to have an exceptional longevity. So, a parent group of around 86 members and they have an average lifespan of 96 years, and the offspring group of around 175 members, and the control group were around 93 members. They found that the population exhibited abnormally high telomerase activity mediated by a mutation in the hTERT which is the catalytic subunit of the telomerase activity.

So, because of this mutation in Ashkenazi Jews, they find that the telomerase is hyper-performing, performing more than usual. So, high telomerase activity credit goes to the

mutation and because of this, when the telomerase is functional, they have a longer telomerase than usual.

So, that is what say normal person have like we saw that around 11 kB is the average link, but in their germ cells, in their somatic like in stem cells, the telomerase activity is so high, it can exceed the 11 kB. So, that even it is a cells are dividing at a normal rate, it will never exceed the so called 4kb length which is a shorter telomerase thing because it is going way too high.

It is just like your starting money is only 1000 rupee, it can easily get exhausted. But if your starting is 1 crore rupee it is it will take longer time to get exhausted, as long as the usage is more or less similar. The 1 crore is there you spend it you know some extremely non-productive thing like 1 crore you put it in overnight you can get rid of 1 crore.

But if you are spending usual as the 1000 rupee fellow is spending, then the 1 crore rupee fellow have a lot of advantage because to exhaust that 1 crore at the rate about 1000 rupee holder is spending, then it will get longer time. So, this is the concept you should have. The telomerase activity when it is high, whenever it is functioning, it will add too much of telomere length. So, that will take longer time to get into shorter length.

So, this research indicates the longevity of the telomerase activity can be connected because the average age of this parent group is 97 years and probably the credit goes to the longer telomeres and they do not show the signs of old age.

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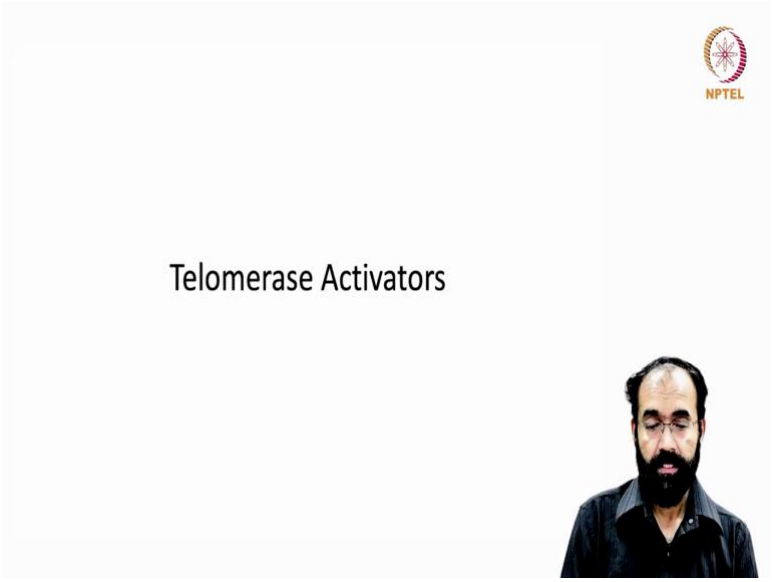

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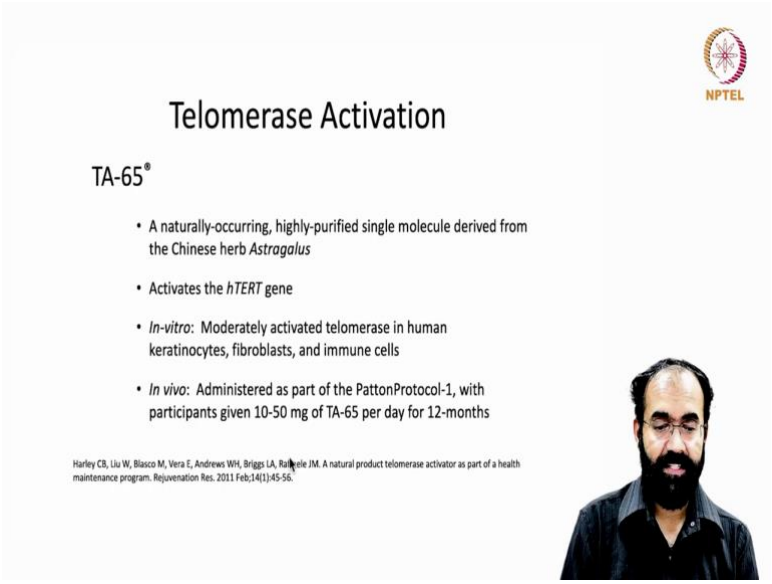

And telomerase activators can be there are many drugs also people have explored. So, a naturally occurring highly-purified single molecule derived from a Chinese herb known as astragalus which is being called marketed as TA-65 and it activates human TERT, hTERT gene that is the catalytic subunit.

In-vitro has shown that the moderately activated telomerase in human keratinocytes, fibroblast, and immune cells, in-vitro study. In-vitro study means (Refer Time: 11:27)

studies is where you culture the cells and you create with this drug, and you see the telomerase is hyperactive which is reflected in the length of the telomeres.

In vivo, that is inside the living animal. In vivo administered as a part of pattern protocol that is the protocol for delivering these drugs which the participants were given 10 to 50 milligram of TA-65 per day for 12 months. And they have shown to exhibit a longer telomeres activity and longer telomeres because of the activation of the telomerase activity.

So, many such you know herbal and a natural occurring compounds have been tried. This does not mean that you can you know avoid getting old, but it can be delayed. Like many research is going on in expanding or extending the life span or expanding the scope of individual cells in a body and beyond their normal life span. So, many such kind of research are going on.

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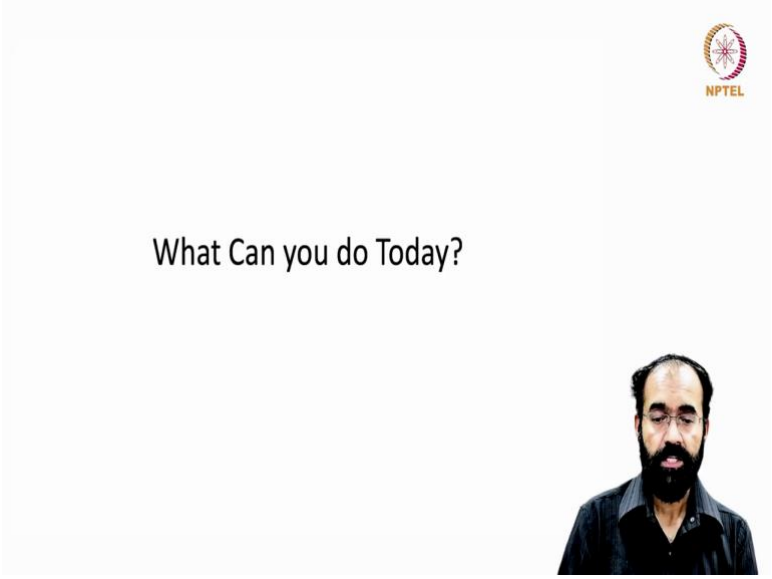

So, let us think what can you do for maintaining a healthy telomere and longer length of telomere due to the telomerase activity.

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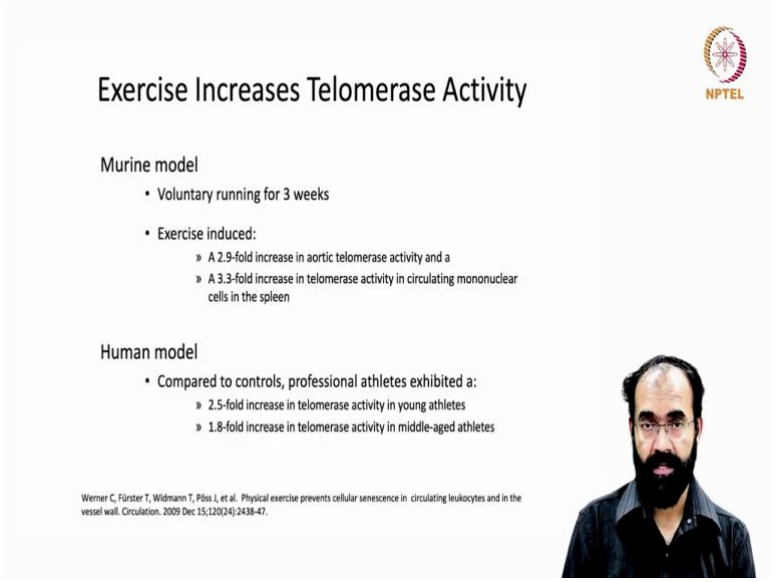

So, exercise is been shown to increase the telomerase activity. In murine model, they have shown that voluntary running for 3 weeks, and this exercise included around 2.9-fold increase in the aortic telomerase activity because when you are running your heart will pump more and you have more of blood supply in the aorta. So, aortic telomerase activity is increasing.

And around 3.3-fold increase in telomerase activity in the circulating mononuclear cells in the spleen that is another observation in that is in mice. So, in human model compared to control groups, professional athletes exhibited a 2.5-fold increase in telomerase activity in young people, young athletes. And 1.8-fold increase in the telomerase activity in middle-aged athletes.

So, what you understand that if you have a healthy lifestyle means exercise, doing exercise and also leading a healthy diet and healthy lifestyle that can have a increased telomerase activity, increased telomere length because of the good or effective telomerase activity can reduce the rate of aging or can increase the life expectancy of an individual cell and probably the aging can be delayed. Now, we will move on to another topic that is about the RNA modifications.

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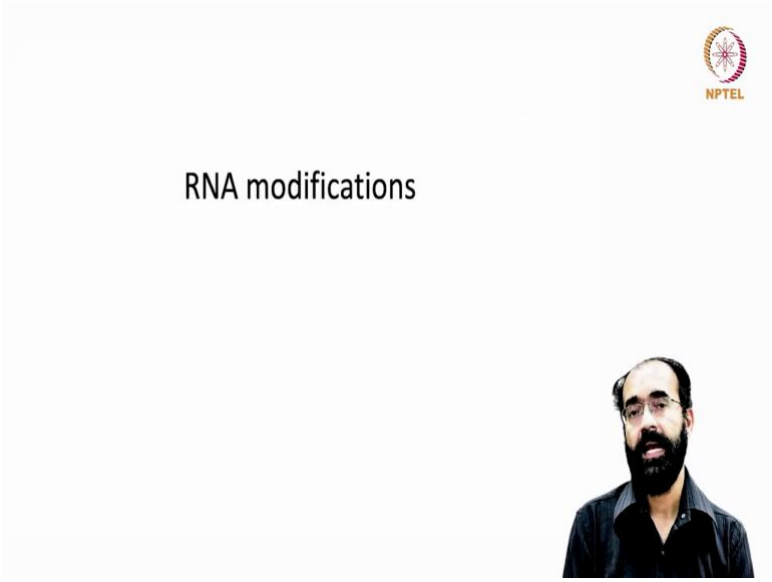

We have seen a lot of RNA modifications, RNA editing, you know we have seen splicing, foliar inhalation etcetera. Now, we are looking into some RNA modification which are not obligatory, that is a facultative. It can change temporarily, it can change with age, it can change with a habit habitat or the food, the drug what you take those kind of things.

So, what you should keep in mind this is something to do with the epitranscriptome that is transcriptome you know you have your genome, genome is transcribed, wherever the genes are to be expressed and we call it as transcriptome. And the genes expression can be regulated by your dietary habit, your environmental habit etcetera. We call it that branch of science as epigenetics. And we call the modification. We have seen some of them like H3K27 acetylation, H3K27 methylation etcetera.

These are all occurring onto the protein attached to onto the DNA and we call it as chromatin modifications. And their modification occurring onto the histone, we call it as histone code. And some modifications facilitate the gene expression, some modifications inhibit the gene expression. So, we are still trying to understand the histone code in human. So, it is now completely understood because lot of dynamic behavior of this epigenome makes the study quite cumbersome.

Many times, this histone modifications we cannot link, whether it is a causative or correlative. Causative link are hard to combine, just because like say you got headache

and that time one crow was sitting in the chair. So, we cannot prove that the crow caused a bird sitting adjacent to you caused you headache.

So, you need to that is a simple correlated; experience of crow was sitting you got headache. But even if it happens 10 times, we cannot establish that it is the causative, crow as the causative agent of your headache. It can 10 times also it can be correlatives that is the challenge we have in understanding the epigenome.

So, these RNA modifications that is occurring not as a requirement, but as a possibility. So, we call such kind of changes as epitranscriptomic changes or we call these modifications as epitranscriptome.

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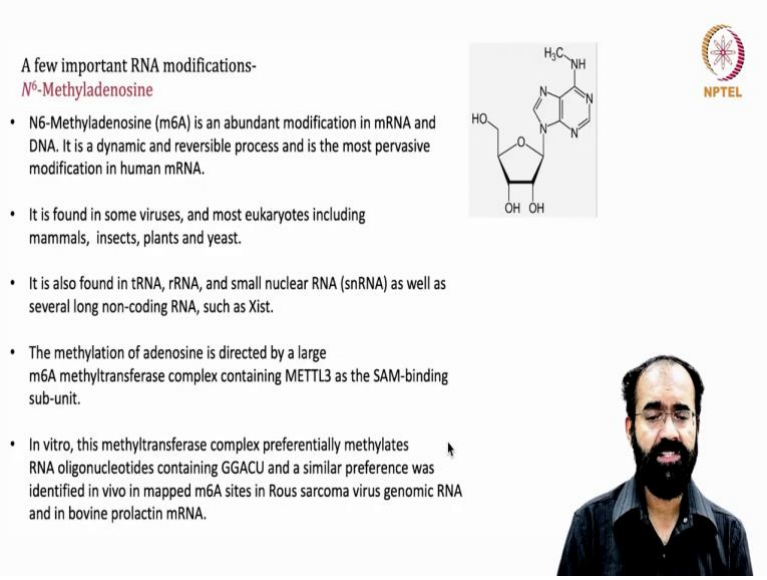

So, one such important modification is N6-methyladenosine, that is a modification of the N6-methyladenosine and we call it as m6A, and which is a abundant modifications in mRNA and also it can be seen in the DNA. And it is a dynamic and reversible process. So, we should keep in mind these modifications are reversible also, reversible process and this most pervasive modification in human RNA.

That means, RNA of any given gene there are so many a residues are there. In some selected residue, it can undergo modification and it can have huge impact in the rate of translation of that mRNA, if it is an mRNA. It can occur onto the non-mRNAs also; that means, RNA that are not just mRNA, other RNA also.

It is found in starting from viruses and most of the eukaryotes have it including mammals, insects, plants, yeast etcetera. Means whichever animal have got an RNA it has the potential to have this modification N6-methyladenosine modification. It is also found in transfer RNA, ribosomal RNA, and small nuclear RNA snRNA as well as seen in several non-coding RNA even such as Xist, X activation specific transcript.

So, the methylation of adenosine is directed by a large m6A methyltransferase complex containing METTL3 as the SAM-binding sub-unit. S adenosine methionine is a molecule that is produced from your liver metabolism. So, in this molecule also have the ability because it acts as a methyl donor, SAM acts as a methyl donor. So, here the modification we are talking about is adding a methyl group.

As you can see here this is the adenosine and all we have we say methyl group that is added onto the N6, nitrogen 6, and this methyl group CH3 is the methyl group that is added. And this modification need a methyl donor and SAM acts as a S adenosine methionine is the methyl donor and the modification occurs on the specific adenosine residues on the RNA.

In vitro, this methyltransferase complex preferentially methylates RNA oligonucleotides containing GGACU. So, this sequence is the GGACU. So, this A can be the target. And a similar preference was identified in vivo also. In vitro means in (Refer Time: 19:53) or in lab condition. In vivo means in living animal. It is mapped as m6A sites can be found in the Rous sarcoma virus genomic RNA and also in bovine prolactin mRNA naturally, to start with initially.

So, what we understand from here RNA modifications such as N6 methyl adenosine, formation of N6 methyl adenosine with the help of methyltransferase enzyme. What is the enzyme? METTL3. So, this enzyme can use the SAM as a donor to add the methyl group onto specific adenosine on various mRNA's. No matter whether it is mRNA, tRNA, it does not matter. It can change the functional properties of that RNA and many times it can be harmful.

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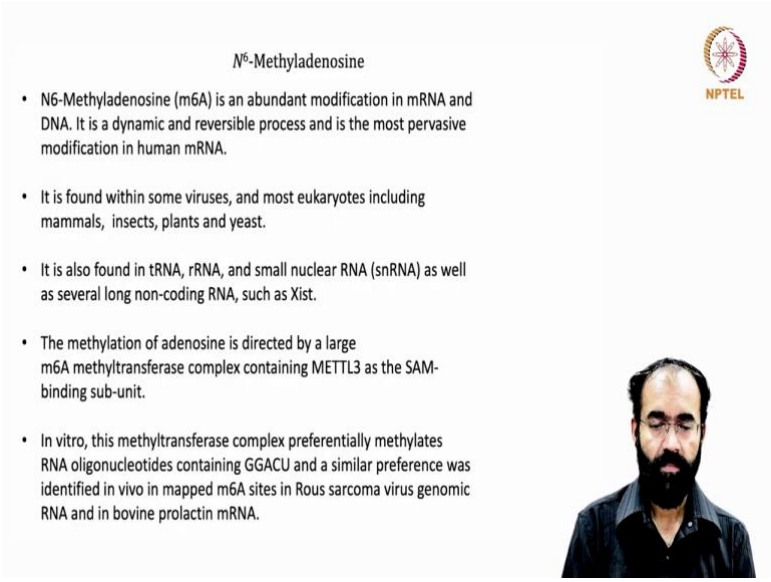

So, N6-methyladenosine is a abundant modification in the mRNA and DNA, and it is seen in various organisms as we speak and they are present consistently across all the species we discussed.

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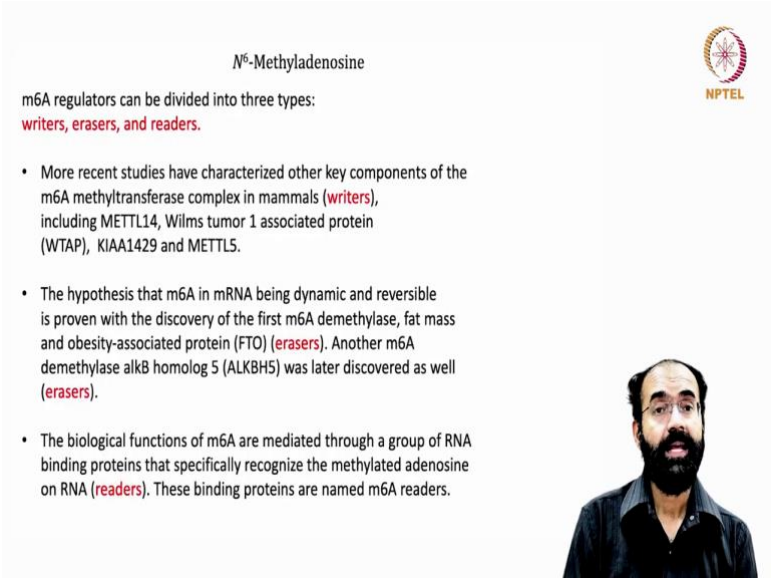

So, m6A that regulators can be divided broadly into 3 category. Like in epigenome also, epigenetic modifiers also, you classify them into these 3 categories. So, this epitranscriptome modified modifications such as m6A regulators can be also classified into 3 groups that is writers, erasers and readers.

Think in this way that in a blackboard or in a greenboard you are taking with a chalk piece and you are writing something let it be ABCD or poem or 1, 2, 3 something. So, someone has to write it in order to have something on the board. Then, someone should be able to read it or someone should be able to erase it. So, you can happen in any of this fashion.

Like, it starts with writing. Without writing no one can read, no one can erase. If nothing is written, what are you going to erase? If nothing is written, what are you going to read? So, writing is the initial step. Then, it has to be read or means it has to be sensed. Here, when you are reading that image will form into your eye and your brain will convert into a signal and your brain will process the reading of that based on what writing you got it. So, this is called reading from a book or from a board.

And erasing means you are just getting rid of that what is written. So, all these 3 molecules are present. That is why it is mentioned that it is reversible. If there is no erasers available, you can never erase it. It is a permanent job. So, it is writer is there, reader is there, eraser is there. So, if the eraser is there, it is working opposite to the writer's job.

So, more recent studies have characterized other key components of the M6 methyltransferase complex in mammals and this is basically the writers, means they create this modification in the adenosine residues. Including METTL14, and also Wilms Tumour 1 associated protein WTAP, and KIAA1429 and METTL5.

So, there are plenty and many more to come because this is one of the recent discoveries in the RNA modification field. So, you will find plenty more coming because as I told you like histone mode itself remains enigmatic although we know a lot, but we do not know completely.

Like that this RNA modification and the enzymes that cost this modification such as writers, will be very difficult for us to comprehend in our 100, more than100 different types of tissue types, even in human model or human organisms, one of the most important organisms.

The hypothesis that m6A in mRNA being dynamic and reversible is proven with the discovery of the first m6A de-methylase. That means, the it is dynamic and it is

reversible simply because they identify a demethylase, m6A demethylase. And this gene is fat mass and obesity associated protein known as FTO and this is a classic example of eraser.

That means, writer adds the methyl group onto that is adenosine and erasers gets rid of that. And there is one more eraser discovered that is m6A demethylase that is ALKB homolog 5 and it is known as ALKBH5. So, this was later discovered to be another important eraser in vivo in various cell types.

So, the biological functions of m6A are mediated through a group of RNA binding proteins that specifically recognize the methylated adenosine and we call it as the reader. So, here we are discussing about the writer, the eraser and also the reader. And these binding proteins are named as m6A readers.

So, what we learned? That, the RNA are vulnerable for modifications, RNA can bring in changes and these changes can be of some crucial impact in the biology of that RNA, means if it is an mRNA's biology is linked strongly with the protein translation. And sometimes an unwanted protein or an over expressing protein can be problematic depending upon which tissue and what protein you are talking about.

So, this is accelerated by writers, and it is decelerated by erasers, and it is sensed by the readers. So, all these three molecules or group of molecules working together will bring in effective changes in the functioning of the RNA.

We will learn more about this RNA modification epitranscriptomics in the next class.

Thank you.