RNA Biology
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Lecture - 52
Telomere, Telomerase and Impact on Genomes: The Importance of Telomeres

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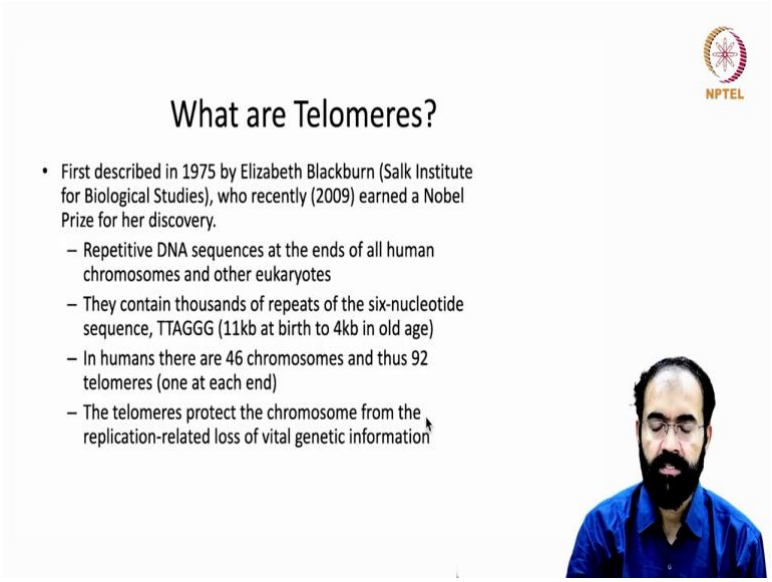

Hello everyone, welcome back to another session of RNA Biology. So, we were here in the previous class that we were learning about Telomeres and we know telomeres are the repeat sequence present at the ends of chromosomes and we can spot them, detect them using some cell biology techniques.

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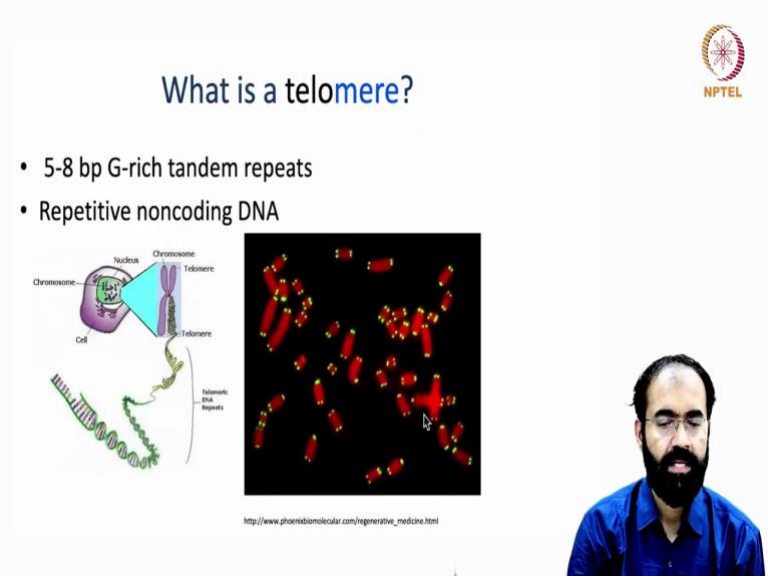

So, telo means end, mere means body, so telomere means end body. It is around 5 to 8 base pair g rich tandem repeat. So, in humans it is T2 A G3 roughly around 6 base in length and it can from organism to organisms it can vary.

So, this is a karyotype where you can see chromosomes and the tips are seen yellow spots and it is a metaphase chromosome where the DNA has divided, but they have not separated yet. That is why we can see each chromosome have got 2 dots on either side. Actually, each chromosome is not one chromosome, it is two chromosomes; one is a original copy and the other one is newly formed. This will separate out in the late stage of metaphase.

So, here you can see this is a nucleus in a cell and you can see the DNA gets compacted in the chromosome structure and if you open it up you can see sequences of DNA that is separated out and we know both the strands of the DNA participates in replication. So, one does a synthesis continuously we call it as leading strand and the other strand is called lagging strand. So, lagging strand makes the DNA copies in pieces because the DNA synthesis happen only in 5 prime to 3 prime direction.

So, in one strand one template strand which is 3 prime to 5 prime direction you can have a newly formed DNA from 5 prime to 3 prime direction exponentially without too much of a problem. Whereas the other strand it has to open up the DNA and then come in a

reverse direction to maintain the 5 prime 3 prime direction because there is no 3 prime to 5 prime growth or polymerization even can takes place.

So, because of this what eventually happen there will be an end replication problem. End replication problem means the fragment that is towards the tip of the chromosome will end up little stunted in one of these strands due to the end replication problem because they will not find a place for the RNA primer to bind because DNA synthesis takes place because of initiation by an RNA primer.

So, when there is no place for the RNA primer to bind then that will lead to an end replication problem because the end will come like if I want to tell you that if a DNA end is from one to say if it is one to 100, so let us for convenience assume a chromosome is 100 bases long. Actually, it is much much it is millions of bases for convenience. So, what will happen? One strand can happily copy from one to 100 without too much of a problem.

Whereas the other strand because of this end replication problem it will not be able to because the lagging strand will not be able to complete the synthesis towards the tip because of lack of space. So, this will so one strand is one to 100 whereas, the other one will be 1 to 90 only. So, this will lead to a reduction. So, the outcome is the parental chromosome was 1 to 100, but the daughter chromosome is 1 to 100 in one strand the other one is only 1 to 90.

So, subsequent generation what will happen? It will go further lower than 90, it will become 80, then it will become 70. So, eventually one chromosome can disappear, so that should not happen right. So, because of that you have long stretches of telomere repeats.

So, the error end replication problem or the reduction in length is occurring to the telomere not to the actual gene or the actual genetic information present in the DNA. That is why at birth we have around 11 KB of telomere, but it becomes 4 KB in old age. So, the shorter the telomere length the older the cells.

So, cancer cells will revive this 4 KB or any shortened one way longer like it can become 11 KB or even more. So, that any amount of cell division will not cause any apoptosis or cellular senescence.

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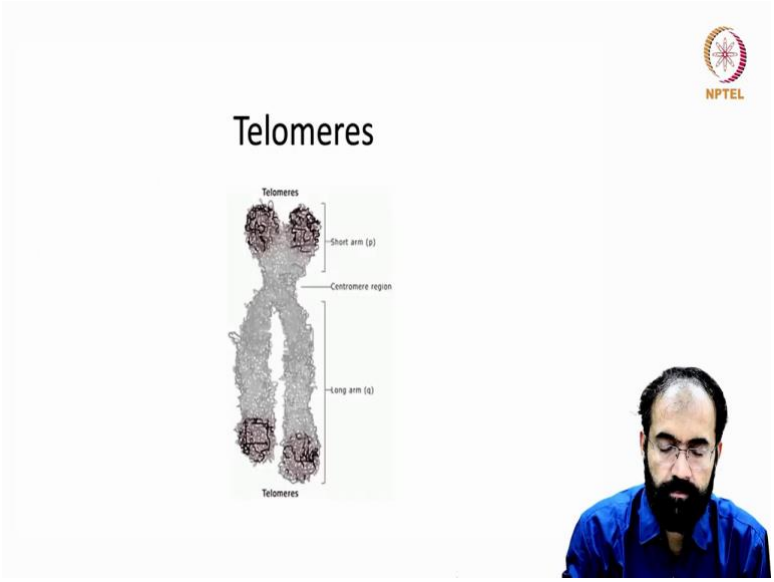

So, you can see here you have two chromosomes and this is the centromeric region this is a short arm and the long arm of the chromosome and the telomere region can be seen densely packed and it as you know the chromosome the DNA is a linear structure, but it is folded and folded and folded like if someone ask you to wrap a rope you will take and roll around your hand and it will look like a small you know a bangle like structure with multiple layers.

So, it can be otherwise carrying a rope will be very difficult for you. So, you will wrap around your hand. Same logic applies when you are when you are binding the chromosome that is why the linear telomere repeat of 11 KB at the young age is now looking like a lump of because this DNA is rounded around.

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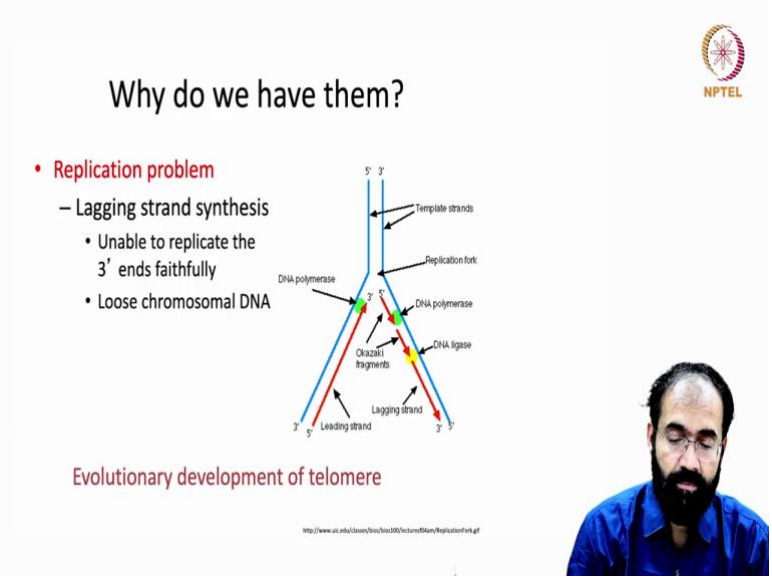

So, this is occurring due to a replication let us understand that what is the replication problem. Why do we have them? So, DNA synthesis takes place in 5 prime, 3 prime direction only. So, you have a leading strand; that means, 5 prime, 3 prime direction, so this is the template what you see in blue color. So, the red color is the newly formed one.

So, it can lead to 5 prime, 3 prime direction continuously nonstop it can go whereas, the other strand cannot go that way. So, it has to open up and start one piece here, one piece here, one piece here like that it has to fuse and the DNA ligase comes in between and fuse it and this RNA primer also has to be replaced by a DNA part which will happen.

So, usually the lagging strand synthesis is unable to replicate the 3 prime end very faithfully because this is the lagging strand and it cannot because it has to synthesize and synthesize enzyme has to hold somewhere, but this is the tip. So, enzyme has to bind on to this template and if it has to make a copy of this it has to bind here in the atmosphere it cannot stand there no it's not like it is just like you want to paint outside the building if you want to work on the tip of the terrace.

So, what they have to do they will build a platform hanging from the roof and you can worker can work on the platform or they will tie a rope around their waist and they hang from the building and paint outside. Otherwise, how you think a human being will be able to paint outside the building if it is very tall if it is only 5 feet tall, they can stand on the ground and paint it.

Same way it to synthesize till the tip of this. So, RNA polymerase is occupying. So, it will synthesize only up to here it cannot complete up to here till the tip if it has to happen RNA polymerase should be standing here in the emptiness that is not possible because it will fall off that problem is not there for the leading strand because it start from here and it continues all the way inside.

So, what we understand the lagging strand often causes the end replication problem. So, they cannot copy the 3 prime end very effectively and this will lead to loss of chromosomal DNA. So, this is the basic need or the necessity of having a telomere because although you are losing it, it is losing from an unwanted portion not the wanted portion because T2 a g3 repeat does nothing special they are basically meant for losing.

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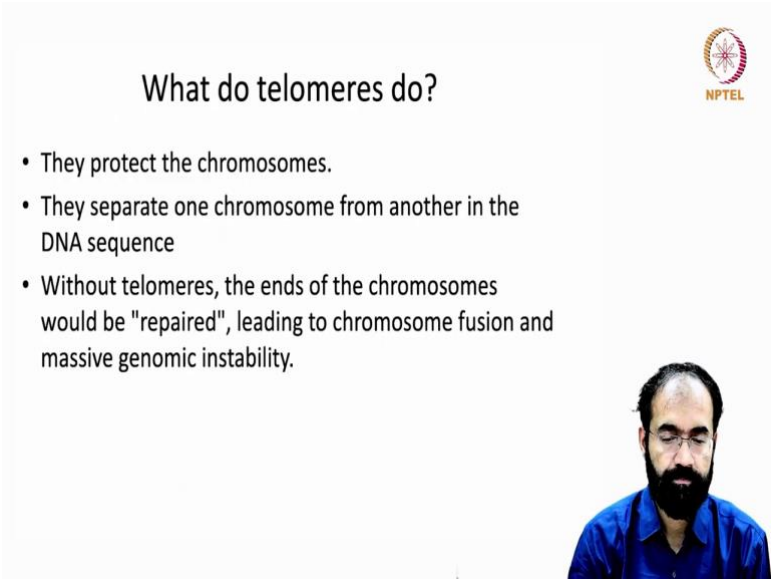

So, what do telomeres do? They protect the chromosome in simplistic form we can say, they protect the chromosome, they separate one chromosome from the another in the DNA sequence. So, prevent chromosome fusion because that also we say you may have heard about the famous example of Philadelphia chromosome that causes cancer that BCR ABL well studied example. So, BCR and ABL fuse because of the chromosomal fusion and it becomes a hyperactive transcription factor that will lead to cell proliferation.

So, I will not go into the detail, but understand that chromosomal fusions can cause cancer also. So, can cause lot of aberrant phenotype you cannot think that every

chromosome is in one cell. So, what is wrong if they are fused together of course, in the first-hand view nothing wrong, but the fusion can lead to loss of gene function sometimes or hyperactivation of a certain genes. So, you do not want chromosomes to fuse for no reason. So, the fusion is prevented because of telomeres.

Without telomeres the ends of chromosomes would be repaired leading to chromosome fusion and massive genomic instability. So, if the repeat sequence such as telomeres were not there then the system will sense it as a damaged portion and it will try to ligate it with another DNA fragment that is available in the cell. So, you end up getting a huge chromosome.

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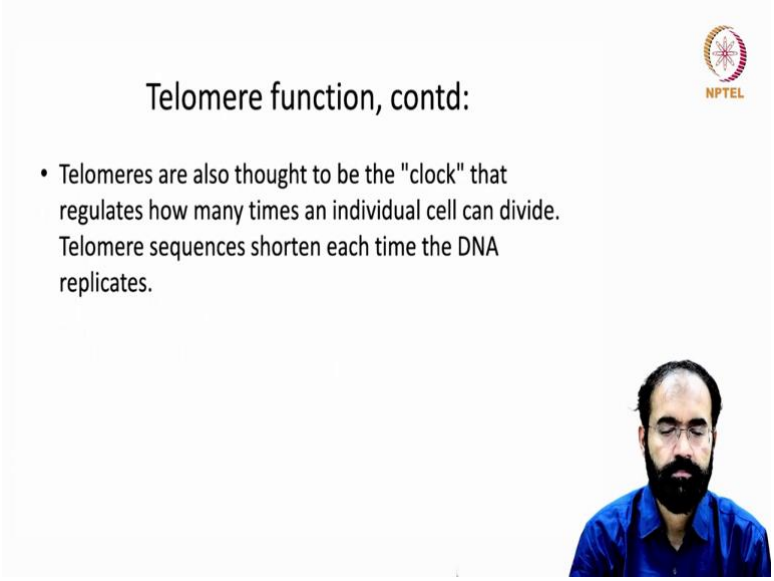

So, telomeres are also thought to be the clock that regulates how many times an individual cells can divide. So, telomere sequences shorten each time the DNA replicates because of the end replication problem.

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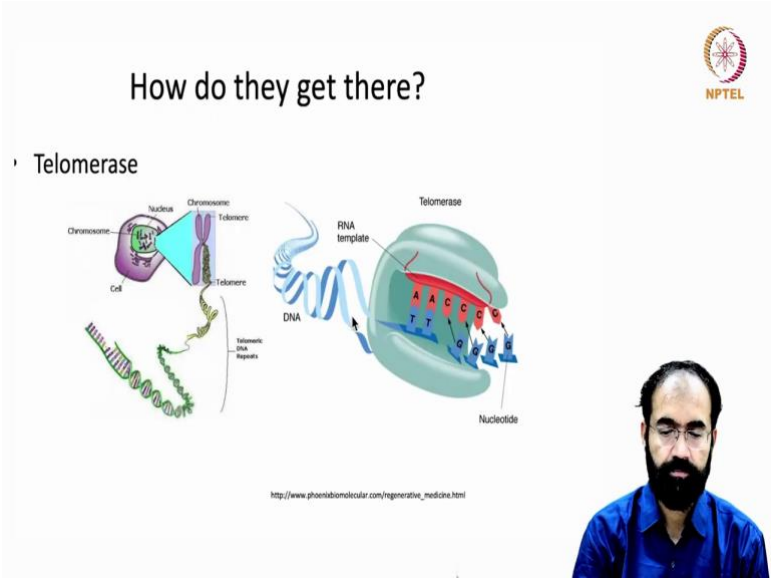

Now, let us understand who puts the telomere onto the DNA how does it come in the first place? The answer is telomerase; telomerase is a RNA protein hybrid. So, telomerase is an enzyme that is capable of renewing or adding the telomere telomeric repeats onto a chromosome.

So, it has a internal RNA template and a protein outside and telomerase is nothing but a reverse transcriptase and telomere is a single stranded phenotype because when it is adding onto a DNA it is adding onto one strand not both strands because to the other strand it is done by another molecule. So, you can see the T2A and GGG the sequence repeats and the template is an RNA template.

So, the RNA template is giving rise to the DNA. So, we can say that enzyme as a reverse transcriptase means RNA is giving rise to DNA.

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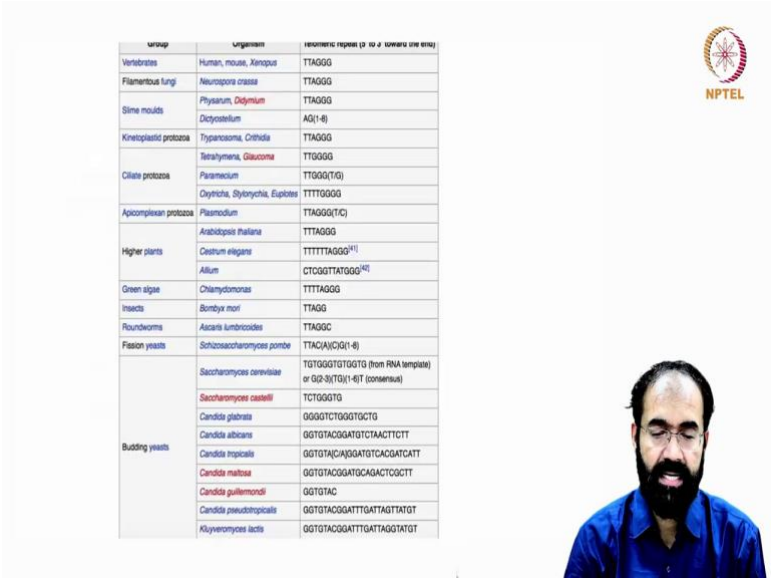

Group	Organism	Telomeric repeat (5′ to 3′ toward the end)
Vertebrates	Human, mouse, *Xenopus*	TTAGGG
Filamentous fungi	*Neurospora crassa*	TTAGGG
Slime moulds	*Physarum*, *Didymium*	TTAGGG
	Dictyostelium	A(G(1-8)
Kinetoplastid protozoa	*Trypanosoma*, *Crithidia*	TTAGGG
Ciliate protozoa	*Tetrahymena*, *Glaucoma*	TTGGGG
	Paramecium	TTGGG(T/G)
	Oxytricha, *Stylonychia*, *Euplotes*	TTTTGGGG
Apicomplexan protozoa	*Plasmodium*	TTAGGG(T/C)
Higher plants	*Arabidopsis thaliana*	TTTAGGG
	Cestrum elegans	TTTTTTAGGG[41]
	Allium	CTCGGTTATGGG[42]
Green algae	*Chlamydomonas*	TTTTAGGG
Insects	*Bombyx mori*	TTAGG
Roundworms	*Ascaris lumbricoides*	TTAGGC
Fission yeasts	*Schizosaccharomyces pombe*	TTAC(A)(C)G(1-8)
Budding yeasts	*Saccharomyces cerevisiae*	TGTGGGTGTGGTG (from RNA template) or G(2-3)(TG)(1-6)T (consensus)
	Saccharomyces castellii	TCTGGGTG
	Candida glabrata	GGGGTCTGGGTGCTG
	Candida albicans	GGTGTACGGATGTCTAACTTCTT
	Candida tropicalis	GGTGTA[C/A]GGATGTCACGATCATT
	Candida maltosa	GGTGTACGGATGCAGACTCGCTT
	Candida guilliermondii	GGTGTAC
	Candida pseudotropicalis	GGTGTACGGATTTGATTAGTTATGT
	Kluyveromyces lactis	GGTGTACGGATTTGATTAGGTATGT

So, you can see the nature of repeats you can see invertebrates such as human mouse and xenopus the sequence is TTAGGG in filamentous fungi neurospora crassa it is TTAGGG. Imagine how conserved it is that even in fungus neurospora crassa and in human it is the same sequence.

You can see slime moulds kinetoplastid ciliates many examples you can see slight variation are there in Arabidopsis thaliana. Instead of TTAGGG it is triple T T2A G3 become T3 A G3 that is the difference between plants like Arabidopsis and human. So, that shows how conserved this whole mechanism is many other fungus you know there can be variations that is not important, but we should know that there is significant conservation exist between eukaryotes.

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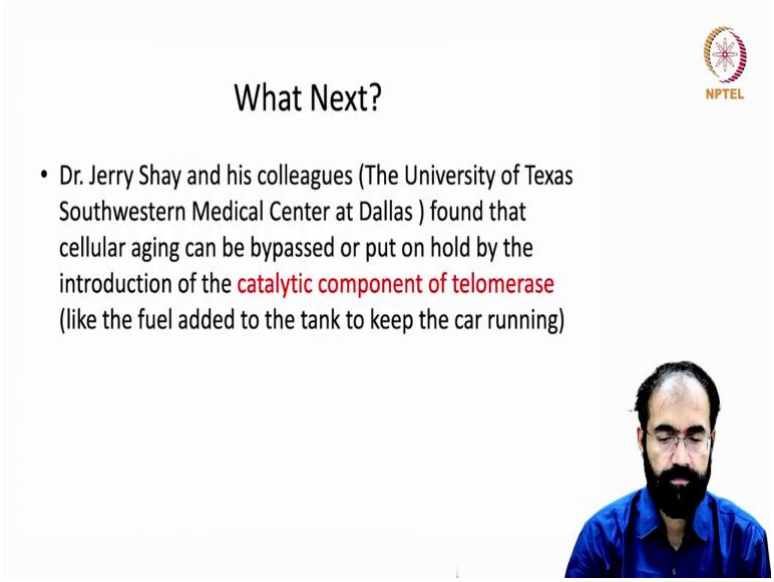

So, Doctor Jerry Shay and his colleagues at the University of Texas Southwestern Medical Center at Dallas found that the cellular aging can be bypassed or put on hold by the introduction of catalytic component of telomerase; that means, adding fuel to the tank to keep your car running as long as fuel is there in your car your car can run.

Like if there is a electricity supply to the motor; the motor will continue to run or any other system you can think as long as firewood is added the fire will not stop fire will continue to burn only limitation will be the firewood. Same way telomerase is the limiting factor, if you have telomerase available the telomere length will not go short no matter how many times the cell is dividing.

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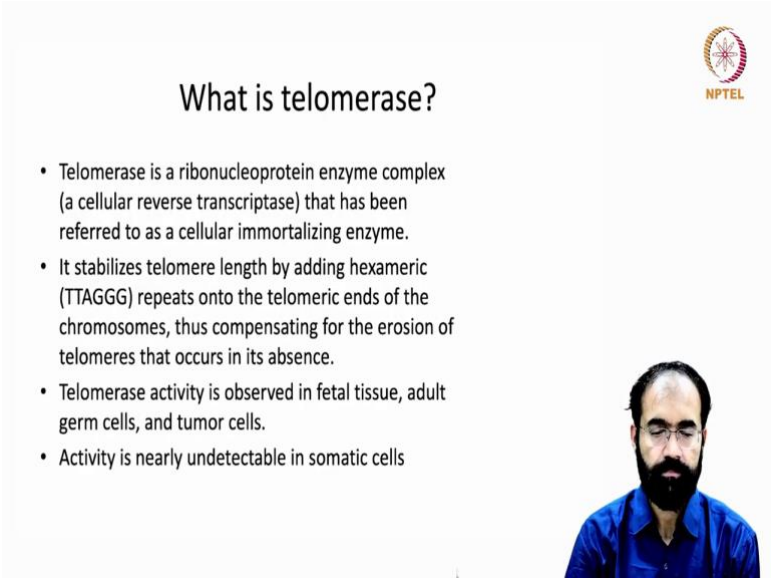

So, telomerase is a ribonucleoprotein enzyme complex and it is a cellular reverse transcriptase and remember this is the one and only cellular reverse transcriptase we have in human body there is no other reverse transcriptase available. So, that is why many viruses bring their own reverse transcriptase to convert their RNA genome to DNA for example, HIV.

And telomerase is also being referred to as a immortalizing enzyme reason being if the telomere lengths are not going to be shortened the cell is kind of immortal it is not going to die at all as it is done in the case of cancer. It stabilizes the telomere length by adding hexameric repeats T2 A G3 on to the telomeric ends of the chromosomes and thus compensating for the erosion of the telomeres that occurs in its absence.

So, the corrosion happens because of end replication problem nothing to do with wear and tear end replication problem happens in the lagging strand because the enzyme cannot stand in the emptiness it needs end DNA strands to hold on if it is holding on it cannot make a copy of the DNA at the place where the enzyme is occupied.

So, telomerase activities observe in fetal tissue and also in adult germ cells and also in tumor cells. Fetal tissue they are rapidly dividing, so you do not want the telomere length to be shorter. So, if you want to know a better all of you might have heard about heard about the famous mammal that is cloned that is dolly it's a sheep. So, it is cloned from the cells of a 6-year-old sheep its (Refer Time: 17:43) cells from a 6 year old sheep.

Now, what happens is these cells are having telomeres of a 6-year-old sheep. So, there is no reviving happens because you have taken the nucleus from a somatic cell where telomerase activity is not there. This one you took the nucleus and put it into the a nucleated zygote. You took a fertilized egg remove the deployed nucleus and replaced with this 6-year-old nucleus from a donor and then the dolly was born.

So, technically at cellular level dolly is 7 years old when it was 1 year old. So, dolly eventually died because of some complications because it is not a fit animal because the telomere started showing its you know old age. So, dolly at the age of 6 years technically it will be 12-year-old sheep. So, it can give the signs of senescence much early in life that can lead to lots of complications.

So, telomerase activity is restricted to fetal tissues and adult germ cells because you have to produce the germ cells to donate to the next generation and the tumor cells evade the stoppage of telomerase activity in multiple ways. Net result is telomerase activity is pretty strong in tumor cells and the activity telomerase activity is nearly undetectable in somatic cell because somatic cells have a uniform way of erosion of the telomeric ends.

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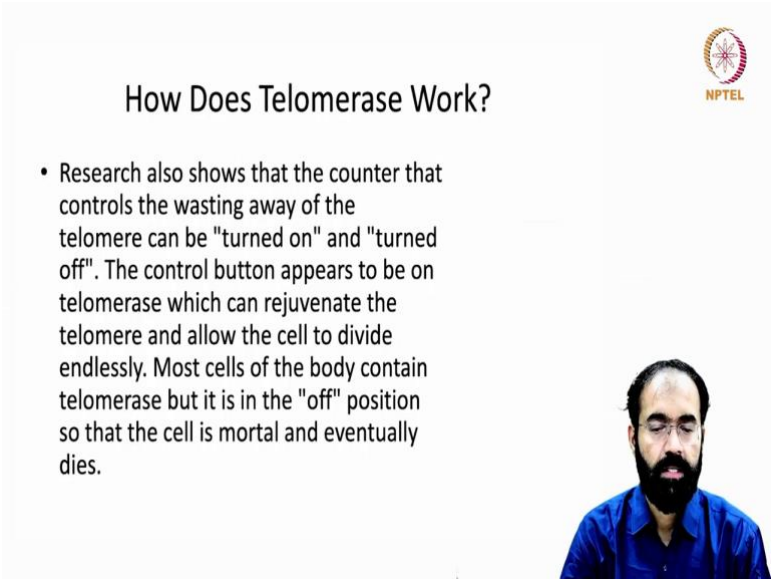

Let us think how does telomerase work. So, research shows that the counter that controls wasting away of the telomere can be turned on and turned off. The control button appears to be on telomerase when it which can rejuvenate the telomere and allow the cell to divide endlessly.

So, cell division is one thing. So, if you if you think in a flip way you can think to avoid getting old, to avoid getting you know senescence cells like old means get wrinkles on face etcetera. Old people you know they will have lots of wrinkles and you know appears in skin and various other things.

What possibly happening in the case of wrinkles is that the cells are not dividing as fast as it should be a to fill a gap and that gap is formed because of too much activity. So, you may have heard about laughing lines. Some people get line laughing lines and you know they have lines on their forehead etcetera because you are constantly bending your skin. So, because of bending there is damage occurring onto the cells and of course, your body repairs them, replace them.

But as you grow old the rate of replacement is not as good as it was in the young age and wherever there is a fold happens on your skin the cells are dying at a faster rate in that dip region or dent bent regions and the replacement is not happening effectively because their telomere is short because they are dividing faster and they are showing signs of old age. This eventually leads to a decline in the rate of cell proliferation and they are not filling the gap and that leads to wrinkles.

So, now we can think if you enhance the lifespan of a cell. Say let us assume your 1 cell is dividing 1 year. It is having a lifespan of 1 year, but what if you give it a lifespan of 2 year by not troubling them like you take enough sleep do not take calorie rich food which will create free radicals or do not take too much of stress or do not screw up your body with any you know unwanted food or any other recreational drugs etcetera anything.

Do not do any damage to your cell then naturally they will live longer and if they live longer, they need to divide less and if they have to divide less your telomere length will not be short. So, instead of 1 year 1 cell life can be increased to 2 year or maybe even 3 years by your own good way of living.

So, this can reduce the rate of getting old, it can enhance the lifespan of the cell and hence you can enhance the lifespan of the organism and the credit goes to the telomeric length. Most cells of the body contain telomerase, but it is permanently kept in off position, so that the cell is mortal and eventually dies.

And if the cell remains immortal like in the case of you know you may have heard about the HeLa cells which is originally from Henrietta lacks, I think there was even a movie a based on that donor of that cell basically that donor live in I think 1970s. So, almost close to 50 years ago these cells lived and the cells and that patient died long back, but the cells are living all over the world in multiple laboratories. It is a cancer cell line I think it's a uterine cancer cell line it's called HeLa. HeLa is from the person Henrietta lacks that is the name of the patient.

So, and that will that cell line will stay forever it is not going to die because it is a cancerous cell it has got the telomerase activity. So, it can live hundreds of years it is not going to die at all. So, many cancerous cells are able to evade the cellular senescence and the mortal features of a normal cell credit goes to telomerase also.

So, do not think that telomerase is the cause of cancer if that is the case a fetal cells and germline cells all should be cancerous that does not happen cancer cells require other requirement, but this is one of the requirements. Just like if you want to fly in the speed of light it's not the instrument your body also should have the capacity to withstand that much of you know speed like you may have heard about the fighter plane there or in rockets.

So, you are not sitting in rocket you are just lying flat and it is thrusting because you do not want the vertical compaction on your body on your muscles. So, your lungs will simply collapse if it goes to that much speed. So, lot of precautions and protection needed otherwise your body cannot withstand that much of pressure.

So, let us quickly see how does this link altogether in the laboratory cells in tissue culture with introduced telomerase have extended their length of the telomeres means telomerase is doing its job they have already divided for 250 generations past the time they normally would stop dividing. So, they can continue to divide and are continuing to divide normally giving rise to normal cells with normal number of chromosomes.

So, if you activate telomerase in a normal cell, you can culture them indefinitely in a petri dish without having any phenotypes of cancer. So, telomerase do not cause cancer, but telomerase is a utilized advantage or feature in cancerous tissue because that is a requirement it's an added advantage the cancer cells have. So, we will learn more about telomere and telomerase in the next class.

Thank you.