RNA Biology
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Lecture - 55
Telomere, Telomerase and Impact on Genomes: Telomeres and Cancer

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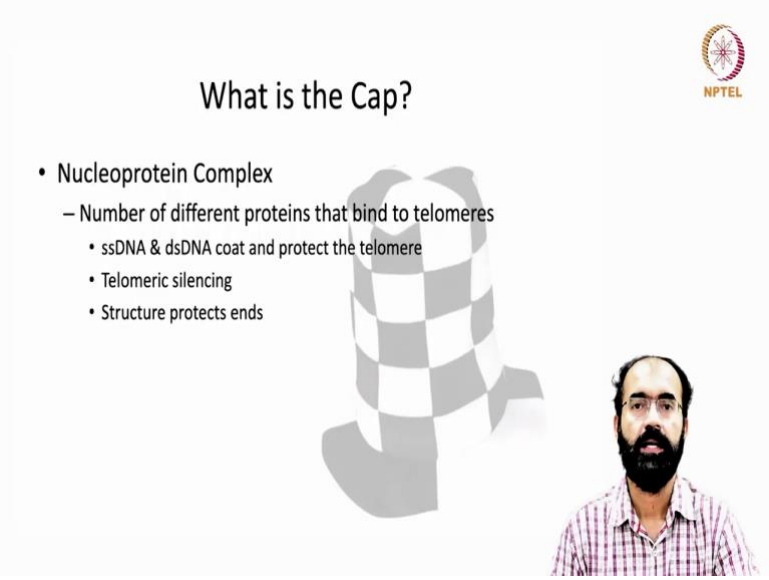

Hello, everyone. Welcome back to another session of RNA Biology. And we were here in the previous class and the telomeric structure forms a cap and it is a nucleoprotein complex made of DNA and proteins. And, it is important in a number of different functions that is in the cell that is the cap is formed from single stranded DNA and double stranded DNA and it forms a coat around the chromosome.

And, it is also important in the telomeric meric silencing means you do not want any gene expression coming from the telomeres and it acts like a protection point from chromosome fusion and broken piece of DNA should not end up in the tips of a chromosome.

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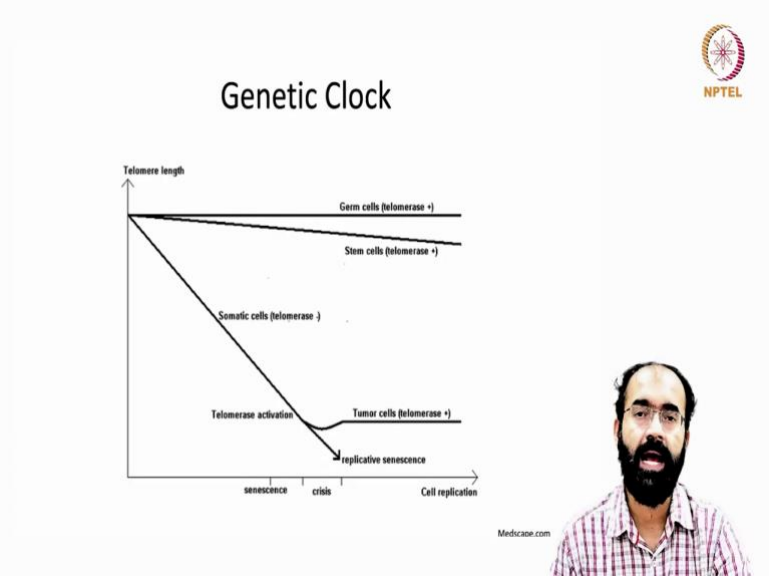

So, now let us understand the role of telomeres as a genetic clock. So, telomere length can be of a definitive length to start with and then you can see situations where the telomerase is present. So, when telomerase is present such as germ cells, stem cells etcetera, the telomere length is more or less going to be on the similar trend that is from bottom 0 to maximum.

This is a definitive telomere length, but in somatic cells, the telomere length decreases as the cell division continue. This is the x-axis is the cell replication. In stem cells and germ cells the number of telomeric length remains more or less the same throughout the lifespan of the organism.

But in somatic cells, somatic cells means body cells the telomerase is missing, telomerase is absent here telomerase is present here telomerase is present, somatic cell telomerase is absent and telomerase absent means the telomeric repeats are lost as the cell division continues.

Once it reach certain number of telomere length and say for here, so, this is the normal length it came all the way down up to here. So, this situation the telomerase can get activated or the telomere length will shorten such that it will reach a cellular senescence and this activation can often lead to tumor cells and they will have telomerase active throughout.

Whereas, in when the cells that is crossing that stage of critical short telomere length, once it has crossed then it will end in a crisis period. That time the cell must die if it is the if the organism of if the cell type has to stay healthy and perfect. If it fails if it fails to do so, then that can lead to very serious problem such as getting into cancer etcetera. So, it will reach a phase of replicative senescence.

So, the cell replication is continuing and the replication cannot proceed further. In some situation it happens such that it will allow the DNA to replicate, but it will not pass on to the G2 phase. It simply cannot go into the G2 phase, this can lead to a replicative senescence means replicated, but it is not going to G2, hence it is not going to the M phase.

Replication of the genetic material is happening, but it is not going further because it is not passing the quality control. And, so, in a normal situation such as germ cells and stem cell the telomerase protects the cell from getting into these crisis and senescence etcetera. Whereas, somatic cell will end up in this crisis and usually the senescence happens in the old age of a organism and animal will not survive beyond that limit.

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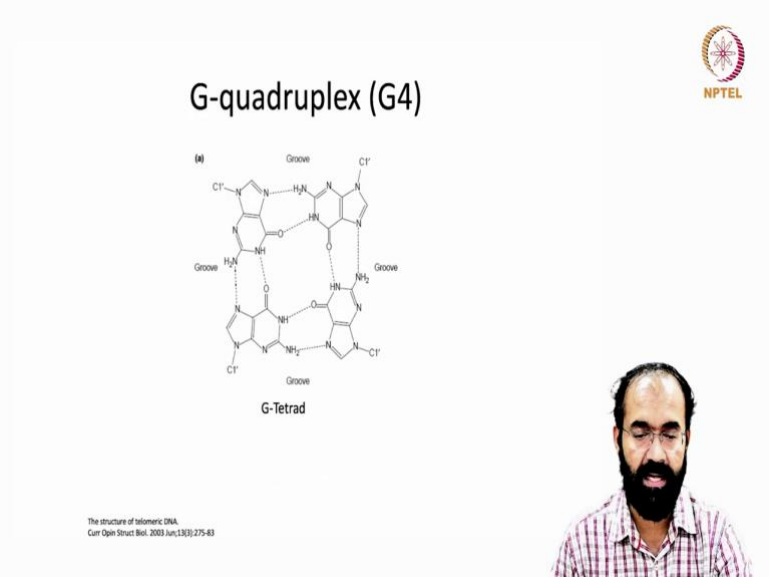

So, the structure what you have been talking about is a G-quadruplex structure, we call it as G4. So, it forms a quadruplex structure formed from the telomeric repeats and this is done through the purine repeat, you can see a purine here, here another purine, another purine, another purine.

So, here the purine is guanine. So, guanine is the one which is a contributing to this quadruplex structure, we know G usually pairs with the C. So, here the G's are bonding each other and it acts like a barrel like structure 1, 2, 3, 4 G's form a tetrad and that will prevent the formation of any erosion. So, the proteins that bind the G4 is stabilizing the structure of the telomere.

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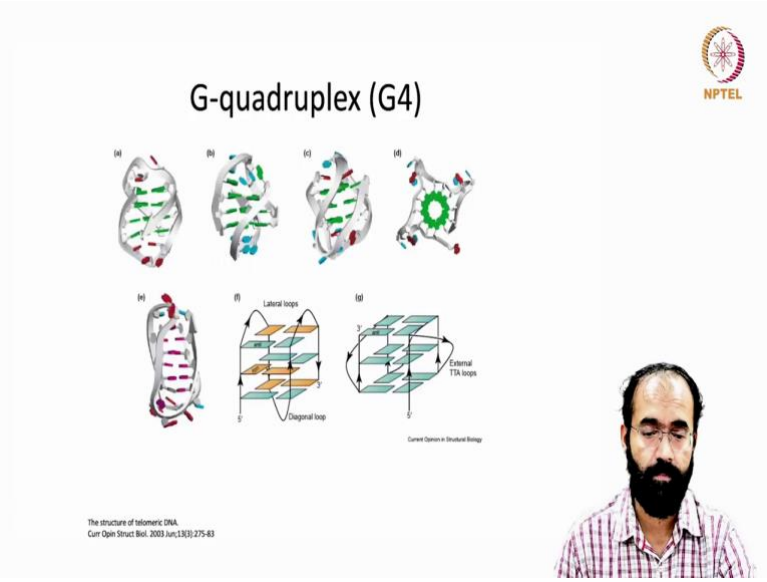

So, G-quadruplex we call it as G4 and it has got different form of maturation and it folds in a unique manner such that the cap, the telomeric cap is very stable and it is never allowed or it will never allow any exonuclease enzyme to come and act. So, it is like a exactly like I told you, it will act like a helmet.

So, this folding and the formation of this unique structure, it is a stepwise manner A, B, C, D, you can see and that structure along with the protein because it is a nucleoprotein complex.

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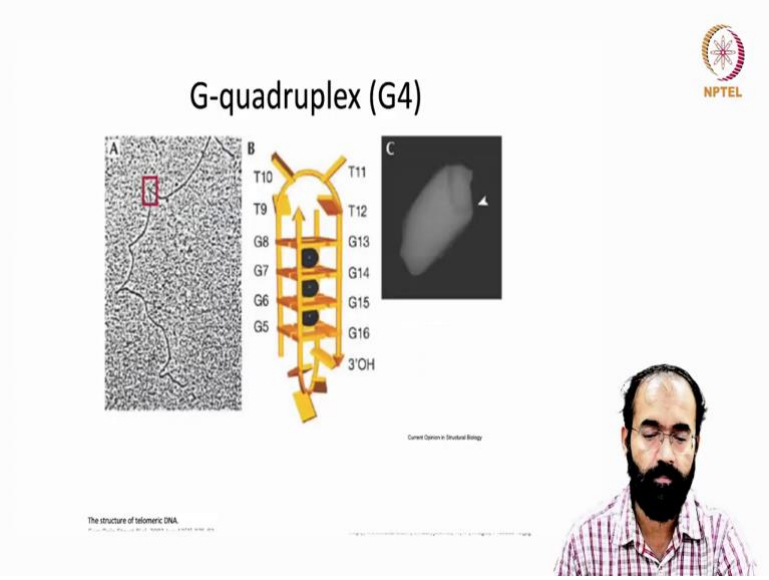

So, G-quadruplex if you look closely which can be visibly seen under an electron micrograph as you can see here and the formation is somewhat like this. The same DNA is folded over and over, it forms a multiple structure of the both these strands and it acts like a multi-storied building kind of structure where these are the individual Gs and you have some specific thymines also T11, T10 and T9 and then G8, G7, G6, G5.

And all these guanosines because we should understand the structure is T2, AG3. So, that is the structure of the telomeric repeats. So, they have the potential to build over and over this quadruplex structure and that will form a very stable telomeric cap.

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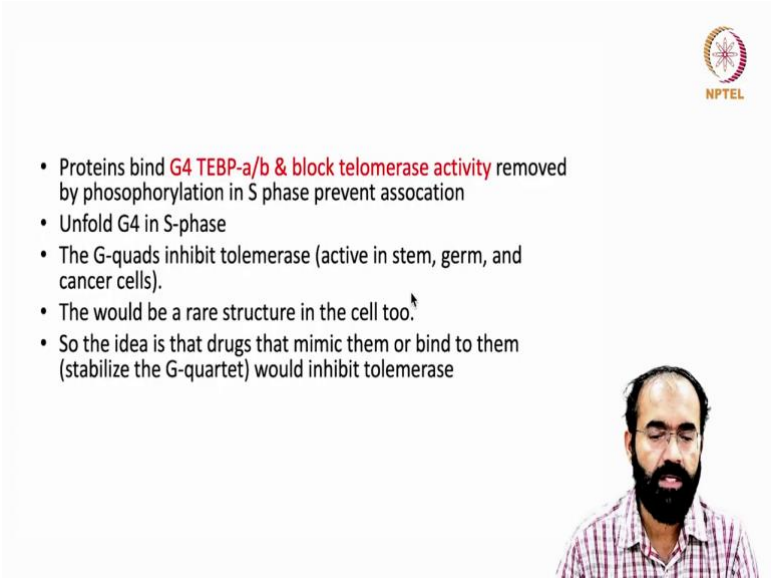

So, the proteins that bind G4, TEBP – a, TEBP – b and they can block the telomerase activity, they are removed by the phosphorylation in the S-phase to prevent the association. So, the proteins that are binding the G quadruplex such as TEBP – a, b and they can block the telomerase activity; that means, telomere structure also contributing or the proteins associated with the telomeric cap also contributing to reduce or prevent the telomerase activity.

So, when the cell replication or DNA replication takes place, the quadruplex structure is lost and the telomeric repeat is lost because of the end replication problem because there is no question of reviving. So, in a cell even if telomerase is available, the G quadruplex can hinder the action of telomerase activity. So, they the G quadruplex G4 is unfolded in S phase that is the synthetic phase of the cell cycle where the DNA replication has to take place.

The G quadruplex inhibit telomerase that is they are active normally in stem cells, germ cells and also in cancer cells. However, the activity of telomerase is vulnerable or the activity of telomerase can be hindered by the G-quadruplex and the associated proteins. So, there would be rare structure in the cell too because it is not in the stem cells and cancer cells, they circumvent this complex structure because they do not need to fear about short-end telomere.

In a normal somatic cell, you need to fear about the short telomere and the chromosome erosion or the chromosome fusion etcetera because they have to live with whatever telomere length, they have got it. So, they have to preserve it. But, whereas, cancer cells and germ cells have the luxury of telomerase hence it can constantly renew it. So, the idea is that drugs that mimic them or bind to them can that can stabilize the G quadruplex would inhibit the telomerase activity.

So, some drug treatment approach can be done to kill the cancer cells. So, you can have some drugs that will mimic in structure to that of G quadruplex. So, that can you block the telomerase activity. So, that cancer cells also suffer from telomeric erosion and possibly undergo apoptosis. But cancer cells can come with newer elegant strategies also that some cancer cells know how to avoid getting apoptosis or programmed cell death.

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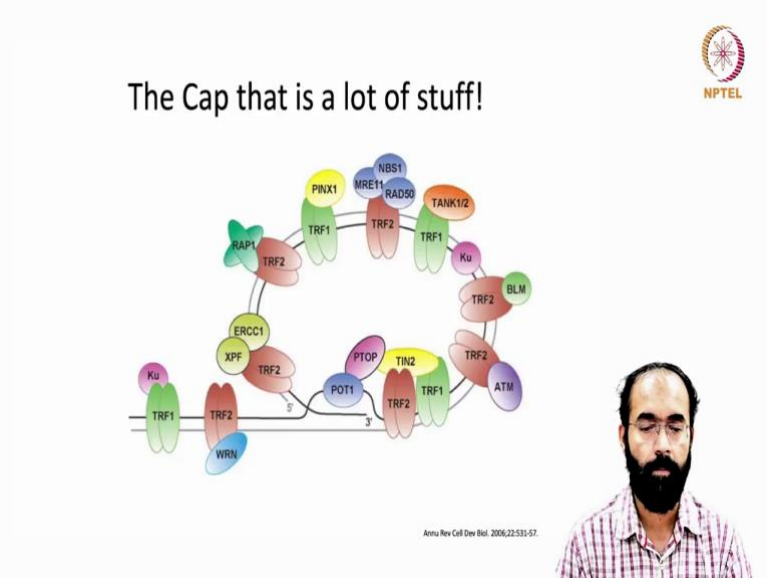

The cap although we say it is a telomeric repeat. It has got lots and lots of stuff especially the proteins. If you see this telomeric cap you can see how many different types of proteins are associated each of the structures are nothing, but proteins. You can see Ku protein, TRF1, TRF2, WRN, protein XPF, ERCC1 like you can go through a lot of proteins they bind together and this is the 5 prime end and it is forming all the way back and the 3 prime end is situated.

So, DNAs double strand this one strand 5 prime end is here and this is the 3 prime end and it forms a folded structure and it forms a very unique and complex structure.

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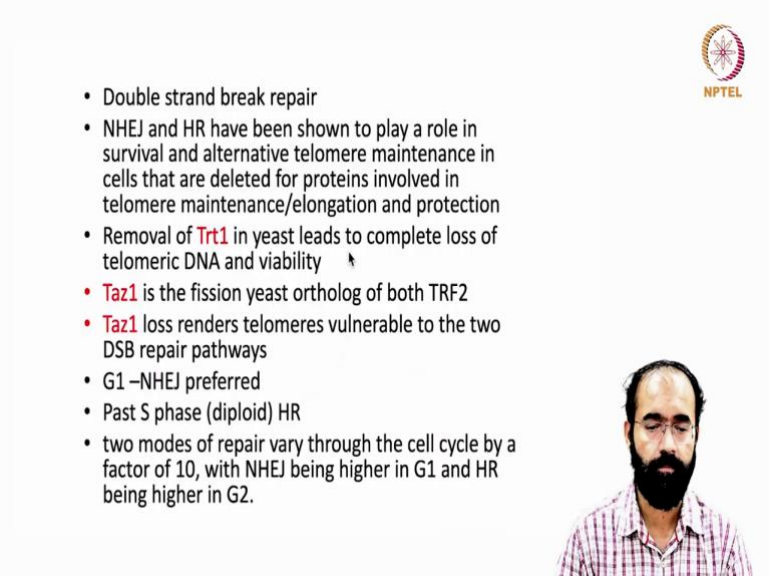

So, they are important the telomeric interactions or telomeric proteins they are important in the double strand break repair; that means, DNA has got a double stranded structure. It can break due to various reason, it can be because of exposure to UV rays, it can be due to some you know some chemical carcinogen exposure etcetera etcetera DNA can break. So, it is not a major problem because you can repair it because with the help of repair enzymes many a times, they are turned on by P53 itself.

But double strand break repair has to be one of the foremost need of a cell if it has to lead a normal life. So, non-homologous end joining and homologous recombination NHEJ and HR have been shown to play a role in the survival and alternative telomere maintenance in the cells that are deleted for proteins involved in telomere maintenance and elongation and its protection.

So, we should understand the NHEJ and HR normally contribute a major role in the survival and also in the alternative telomere maintenance. So, sometimes NHEJ is a necessary for breaking the damaged DNA. But if the breaking has happened in a gene part, then NHEJ often results in a loss of information.

Same with homologous recombination also; many a times homologous recombination if it happens in the identical region there may not be a major loss like we saw that homologous recombination in intron gain and intron loss situations. But if there is a

damage to an existing gene then there may not have a adequate protection from any source whatsoever possibly cannot happen.

So, the organism or the cell or a that given gene suffers the only way of rescuing is if that particular gene is not going to be expressed in the tissue, then that cell may not have a major problem to start with at present. But, if that defect happens in the germ cells for example, then that will be passed down to the next generation and that next generation will suffer for sure.

So, removal of one of this protein that is Trt1 in yeast cells to complete the loss of telomeric DNA viability. So, if this protein Trt1 in yeast if it is damaged it leads to complete loss of telomeric DNA and the viability of the cell. It is just one protein that binds to the telomeric repeat. Another protein Taz1 that has been studied in the fission yeast which is a ortholog of TRF2.

And, Taz1 loss renders the telomeres vulnerable to the two double strand break repair pathways. Means the DNA repair pathways are affected very badly means it will be vulnerable to the double stranded break repair. If there is a double strand DNA damage, I told you that it needs to be repaired whether it is through HR mechanism or NHEJ mechanism. So, double strand repair pathways become vulnerable, if the Taz1 gene is lost.

And, at the G1 phase means the beginning post mitosis is a newly formed or newly born cell is in G1 phase. So, NHEJ is preferred at that stage and it passed the S phase say for example, G1 then S phase where the DNA is replicated and then it goes to the G2 phase. So, once it crossed the S phase at deployed cell stage because after S phase in G2 phase the genome is deployed, genome is too copied because the entire chromosome set is divided now in G2 phase and later M phase it separates out.

So, in G2 phase every cell will have double like if a normal human have got 46 chromosome and it is 92 chromosome in the G2 phase and in M phase that separates out into 246, 46 bearing cells. So, once it passed the G2 phase then HR is preferred a reason being very simple because you have a copy to make to comparison to like because in S phase it already replicated.

So, you have a DNA damage, then it has a template to look after which copy you should be making. So, homologous recombination is preferred, past the S phase. So, two modes of repair vary through the cell cycle by a factor of 10 with NHEJ non homologous end joining being higher in G1 phase and HR being higher in G2 phase means this does not mean that the other mechanism do not come into picture. That is not that past S phase NHEJ cannot happen technically can happen.

So, what a cell prefers, same way in G1 phase homologous recombination also can happen, but availability of a normal template and getting access to it can be limited because of deployed state of the cell; whereas, in G2 phase it will be tetraploid stage of the cell which will eventually become diploid after the mitosis.

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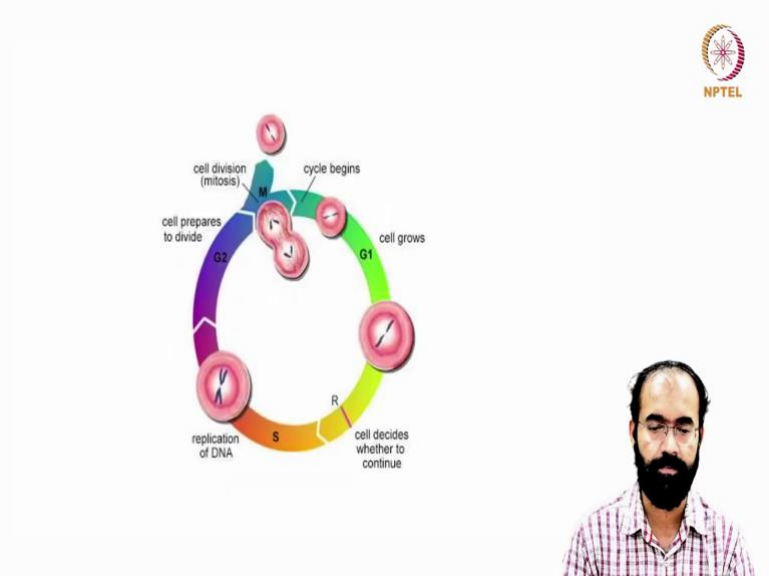

So, this is a cell cycle which is just for your understanding. This is the mitosis phase where the actual cell becomes two and this daughter cells immediately enter G1 phase and in that phase the cell grows. And, if the nutrition and other signaling events go smooth then it will get into a S phase and before that there is a critical phase that is called R phase or restricted phase.

Cell decides whether to continue into S phase or not. So, that is a it is just like in a human life all of you who are in your youth decides at what age I should get married. It is a very determined some people say that ok I need another few more years to get married or some people say no I am ready to get married.

So, it is a critical age, some people say decide ok no I want a job before I can get married; some people say no I want to complete my studies in abroad so and so university before I can get married. So, it is a decision which is taken at individual level. So, every cell which tissue you are talking about it has got this restrictive phase where it decides whether to go into S phase or not because once it gone into S phase it is a point of no return it cannot reverse back.

G1 phase it can leave it is rest of its life without too much of a problem. Every cell in your body currently which is not dividing is in G1 phase. If it has come out of the cell cycle, we call it as G0 phase. So, if there is a requirement then this G0 cells or which has come out of the cell cycle will now enter the cell cycle. So, it has to start from the scratch G0 cells now, become G1 and then S and then G2 and then M phase M is for cell actually dividing.

So, this G2 phase replication phase happens in the S phase and G2 phase cell prepares to divide. So, this G stands for growth basically. So, this is a growth 1 and this is growth 2. So, growth 2 has nothing to do with the homeostasis function, that growth 2 is mainly preparing the cell for doing mitosis. So, requirement gene expression protein production etcetera happens that is with one goal in the mind.

Like if you decided to go for a long tour a foreign trip your whole preparation will be for towards that at least towards the last 1 week or 2 weeks. You will not be buying vegetables fruits etcetera for your fridge or at home you will not buy too much as you usually do because your whole preparation will be some buying some snacks what is required for a long trip travel etcetera. Same way the G2 phase will be preparing the cell mainly for M phase to manage the M phase.

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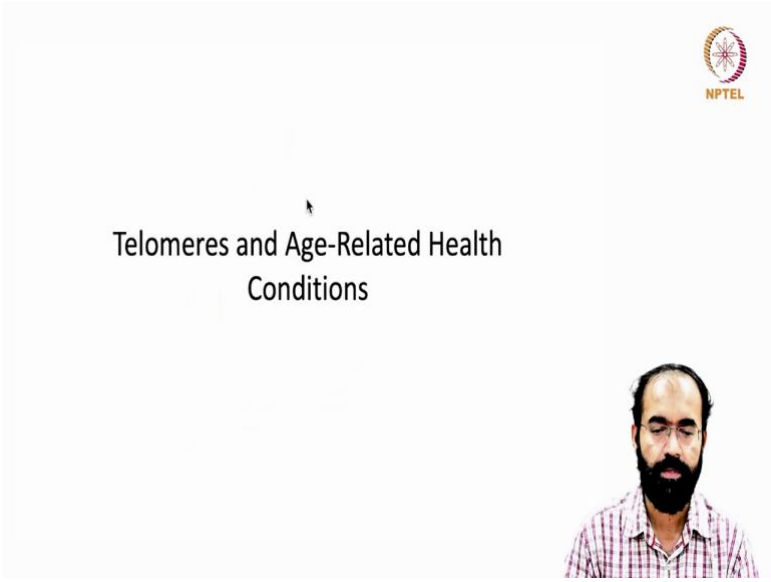

So, telomeres and age related health conditions, it is something important for us to understand about them.

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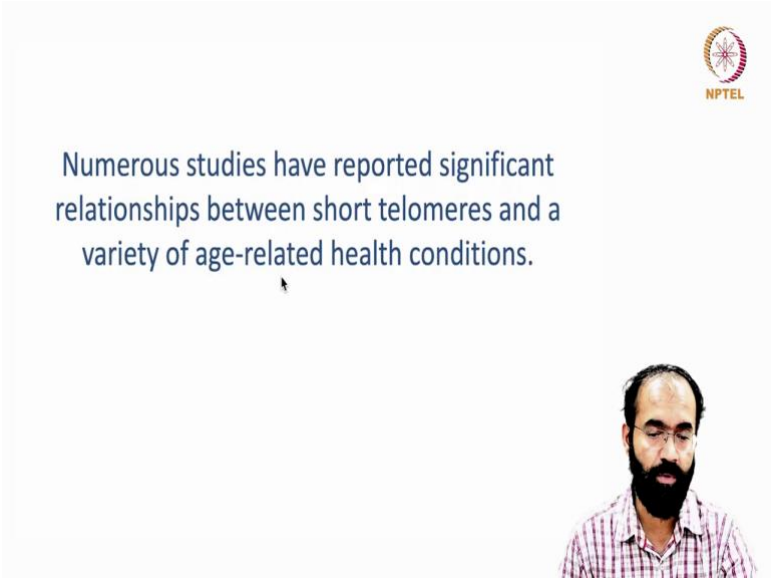

Numerous studies have reported significant relationships between short telomeres and a variety of age-related health conditions. So, what are they? Let us see in a quick manner.

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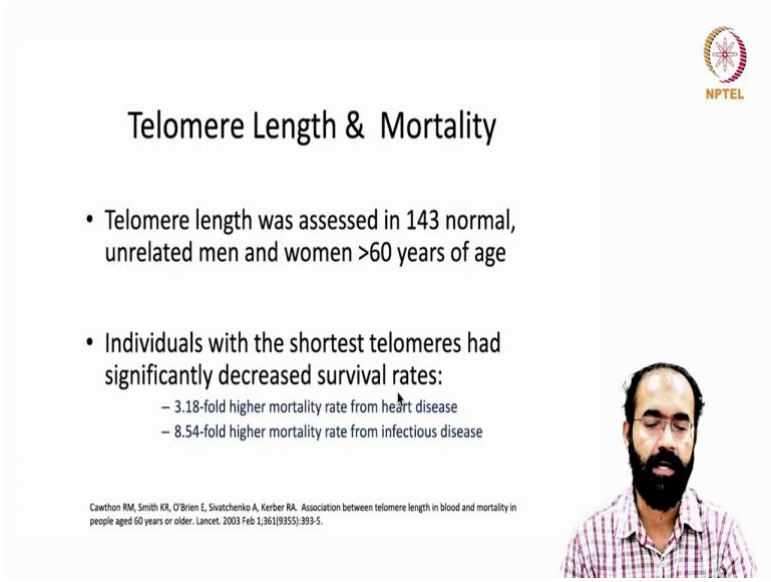

So, telomere length and mortality is a solid connection or a strong interrelationship is there for mortality of a cell or even an organism. So, telomere length was assessed in 143 normal unrelated men and women who are more than 60 years of age means basically they are in the post second half of their lifespan. Individuals with the shortest telomeres had significantly decreased survival rate say, 3.18-fold higher mortality rate from heart disease; 8.54-fold higher mortality rate from infectious disease etcetera.

So, in this many numbers of human studies where you simply collected the tissue and analyzed their telomere length only thing is their calendar age was more than 60 years. So, this enhanced the by 3-fold in the case of heart cardiac disease and 8-fold in case of infectious disease.

So, what is important is if your telomere length is getting shortened and shortened, you are vulnerable to either a metabolic disorders such as cardiac problem or infective disorders like some infections such as COVID or maybe other bacterial infections.

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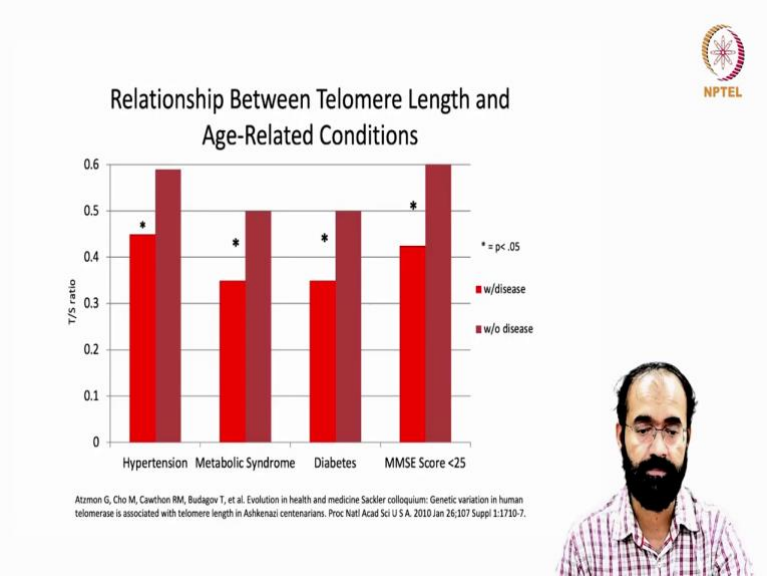

So, let us see the relationship between telomere length and age-related conditions. So, there is a ratio called T/S ratio we will discuss about that in detail. So, let us see an example. So, this is a disease hypertension and metabolic syndrome and diabetes and MMSE means various infectious score etcetera. So, P is the significance level, if the P value by T test or P value is less than 0.05 it is considered significant. So, that is what you give a star.

So, you have two categories one is with disease and without disease. So, the telomere and this somatic T/S ratio basically the length of the telomere to rest of the chromosome length. So, if the T/S ratio is high that indicates that telomere length is short because telomere length divided by somatic rest of the chromosome length. So, you are making it like a proportion. So, if the telomere length is short naturally this value will go different.

So, as you can see here this has a telomere length divided by the rest of the chromosome the somatic length and then you will end up having in with the disease and without disease two category, you say disease having with higher T/S ratio is a significantly accepted fact. So, you can say that as the T/S ratio goes higher and higher, you end up getting a more chance of having disease.

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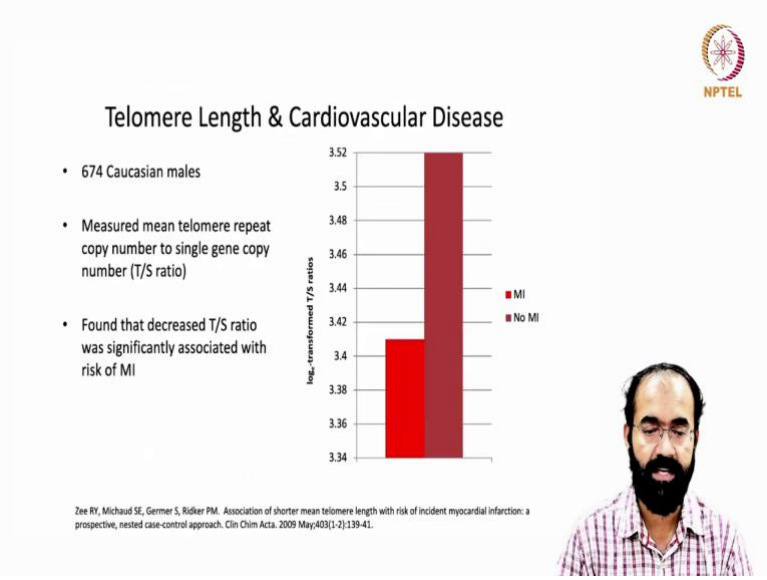

So, now let us see telomere length and cardiovascular disease. It is done in 674 Caucasian males. It is measured mean telomere repeat copy number in a single gene copy number it is a T S ratio. So, it is measured T S ratio basically is mean telomere length means you cannot measure an absolute telomere length because you take the total length divided by number of chromosomes.

And, to a single copy number; that means, what is present in a given chromosome. So, that is what you are comparing as a TS ratio. So, it is found that the T S ratio was significantly associated with risk of myocardial infraction that is cardiac disease diseases. So, you can see that this is represented in terms of log value like T S ratio in the y-axis.

You can see here as the length of the T S or the T S ratio is lower you can get a higher chance of myocardial infarction and if the T S ratio is larger then it can decrease the chance of having myocardial infraction. And this is also connected with the age.

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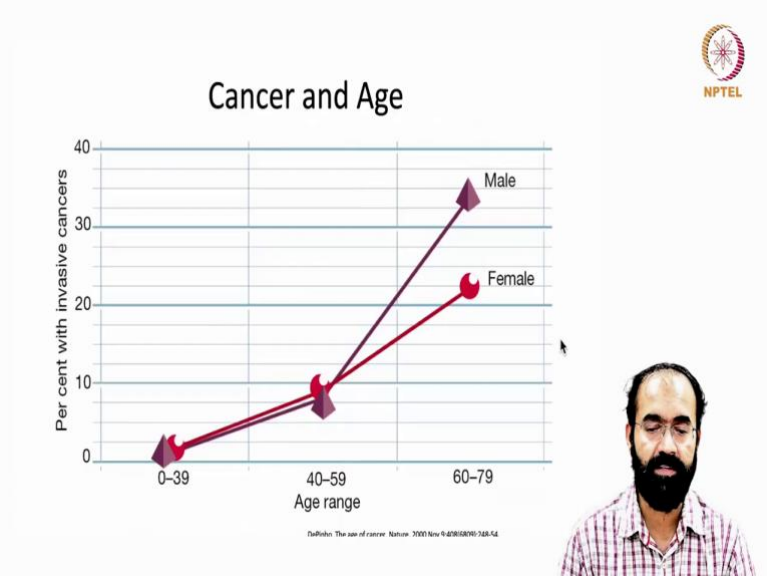

So, let us see we all know that cancer is a situation that is in the second half of your life as you cross age 50 or something you will be vulnerable more for cancer. This does not mean that children do not get cancer etcetera, but you will become more vulnerable for cancer. Credit goes to your weak immune surveillance etcetera. A mad cell should have been killed or sensed by your immune system or should have been killed by apoptosis etcetera.

So, here you can see percent with invasive cancers this is the 0, 10, 20, 30, 40 percent like that in male and female individuals and this is the age in x-axis 0 to 39, 40 to 59, 60 to 79. So, as you can see here age have got a strong connection with the cancer. The rate increases and more vulnerable to males than females and of course, it has a lot to do with hormones also. Estrogen have got lots of protective effect; estrogen and progesterone compared to testosterone.

So, these are all some of the facts, but age have got a strong implication in the telomere length also. So, we will study more in detail about the connections of telomere length and the onset of cancer and how telomere is influencing similar to that of myocardial infarction or heart diseases, how telomere length is affecting the cancer progression etcetera, we can see in next class.

Thank you.