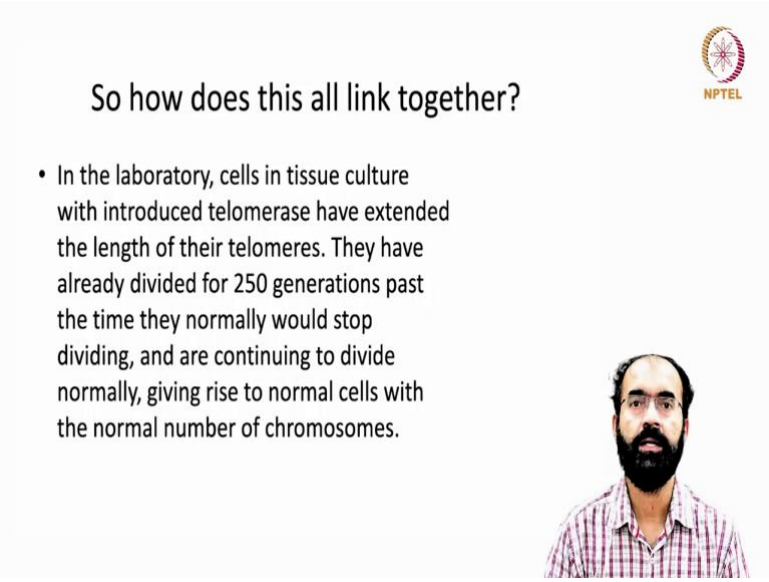
RNA Biology
Prof. Rajesh Ramachandran
Department of Biological Sciences
Indian Institute of Science Education and Research, Mohali

Lecture - 53
Telomere, Telomerase and Impact on Genomes: Telomerase and Aging

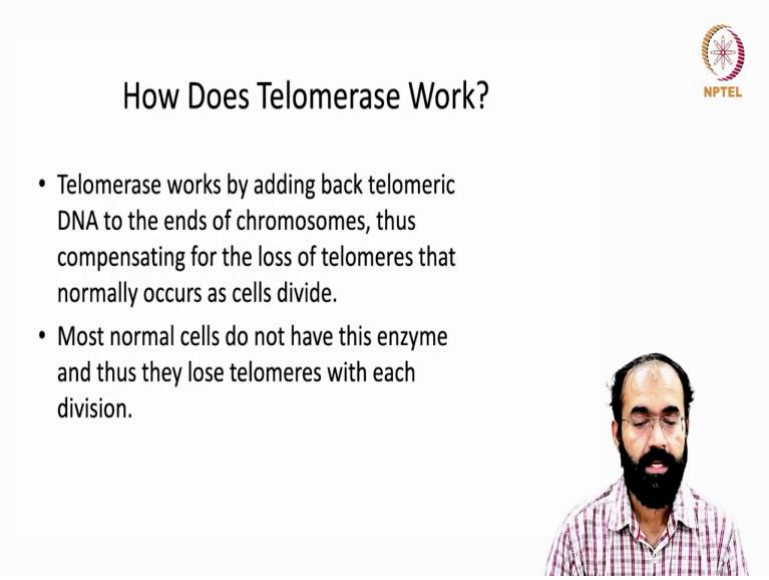
(Refer Slide Time: 00:21)



Hello everyone. Welcome back to another session of RNA Biology. So, we were here in the previous class and we were learning the importance of telomerase, and also finding experimental evidences to support that what is the implications of telomerase and can we prove it that telomerase indeed is important to extend the longevity of a cultured cell in tissue culture.

So, if you over express the telomerase, we can see that the introduced telomerase can extend the length of the telomeres and they can divide for several rounds. Like examples of 250, 300 generations they can passed, but still, they do not have any abnormal features, such as cancer. So, for a normal cell, if you want to express or if you want to maintain them in a actively proliferating state, then you need to have telomerase available otherwise the cells will start showing signs of senescence or signs of old age.

(Refer Slide Time: 01:38)



So, how does telomerase work? Telomerase works by adding back to an existing array sequence. It do not start from the scratch. The T2AG3 sequence is already there and telomerase will keep renewing it. Just like you renew your you know vehicle insurance or your house insurance or medical insurance something like that. You already have it and you continue to renew it. That is exactly what telomerase is doing.

So, it keeps keep it is work by adding telomeric DNA to the ends of chromosomes, thus compensating for the loss of telomeres that normally occurs as the cells divide. So, most normal cells do not have this enzyme and thus they lose telomeres with each division. We saw in the previous class that, around 11 kb long telomeres at the young age, embryonic stage now eventually become 4 kb in the old age. So, and also you should understand not every chromosomes have equal length of telomeres.

And this is something important you should understand, not that every telomere will have the same length and their rate of decline also will be at same length. So, it can vary from cell to cell. If a cell is actively dividing, a given tissue is actively dividing then the chances of that particular cell or that particular cell in a given region.

Say in your face, your face have got so much location. But some area there might be constant wear and tear might be happening. So, this can lead to some malfunctioning, malfunctioning means not cancer. So, like you can see some part of your body will have dry skin or some part of your face will have dry skin, but not everywhere.

So, one can always argue, oh that particular area the sebum production or the sebaceous gland is not active. So, we should understand the dry skin is a sign of not functioning cells properly. So, the sebum production is not working as efficiently as it happens in the neighboring cells.

So, hence, you will see some part of your body's having dry skin. Same way some patch of your body may be little bit rough skin, some patch will have you know little bit wrinkles more than the others. So, in a given location if you are seeing, not two cells will be eroded or they are not damaged equally and this can reflect in their telomere length also.

So, since each chromosome do not have the same length of telomere, which chromosome reach the threshold the lower threshold that can trigger a senescence or old age or sign of old age response in that a particular chromosome onwards and that will reflect in the entire cell.

So, not that every chromosome should have extremely short telomere, but their rate of decline in a given cell will be more or less same, because whenever a cell is dividing it cannot escape one chromosome from dividing or it cannot keep aside one chromosome from dividing.

So, every chromosome has to divide, so the rate of loss also will be same. But in a given tissue, if there are 100 cells, we cannot assume all the 100 will have say chromosome number 1 in all the 100 cells have got same length. It would have been same to start with at the embryonic stage, but eventually the length is decreasing.

So, also one more thing in some animal such as mice the telomere knockout telomerase knockout is not going to give a serious phenotype, until F5. There was an interesting paper published in the journal cell few years ago, that clearly showed demonstrated that the sign of old age you start seeing in F5, means filial generation 5, means parents giving rise to the young one kids is F1 parents are the founder population F1 and F1 giving rise to next generation is F2, and F2 giving rise to next generation is F3.

Like that, it have it will reach up to F5 for the mice to start showing signs of old age in a telomerase knockout background. It simply because mice have got disproportionately high telomere length so, the telomere even though the in the germ length cells, it is not

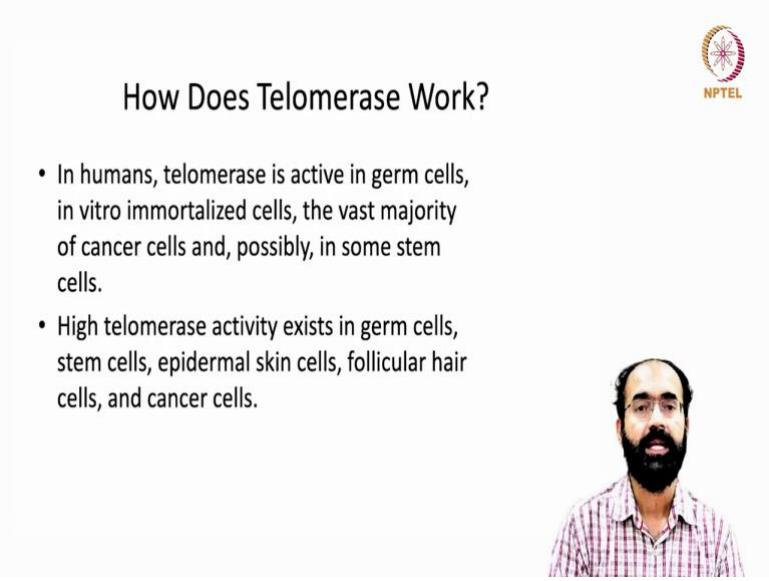
being rejuvenated as it happens in the wild type, because here we are talking about the knockout mice means telomerase is knockout.

So, it takes up to F5 to start showing signs of senescence like you know distinctive, you know skin wrinkles, you know hair patches of hair loss etcetera. There are different phenotypes to indicate the cellular senescence and that mice will look like old. So, you may have seen about there is a Hollywood movie A Curious Case of Benjamin Button, that all they also talk about this you know the kid is born like old, and then slowly it moves of course, it is a fantasy movie.

And, but there are it happens in reality also. But it does not grow backwards in that movie the character is growing backwards to become too young. That does not happen. But there are cases humans who will be born really really old and the cause are something else to do with the genetics nothing to do exclusively with the telomere length.

What I am saying is having old age look is possible in human beings also in some peculiar disease conditions. So, telomere length is very important to maintain healthy nature of that particular organism and the tissue type. So, most normal cells do not have this enzyme and thus they lose their telomeres as and when the cell continue to divide.

(Refer Slide Time: 07:53)



In humans, telomerase is active in germ cells and also in vitro immortalized cell. Immortalized cells means you have different ways of immortalizing a cell. Immortalizing simply means you can pass on the cell culture for generations. We usually refer the word passage.

So, a culture of cell when you are passing on to one generation, you took a frozen (Refer Time: 08:18) from a freezer and you revived them in petri dish and that is called one passage. And then you are again inoculating that is second passage like that. So, this immortalization is possible by transfecting with some specific types of viruses etcetera.

Basically, you are deregulating the normal say in a cell that normal cell have got a distinctive way of cell division. Like we usually refer to them as primary culture say you took a organisms a given tissue, and you separated the cells and allowing to grow in a petri dish that is called primary culture.

Primary culture cannot grow more than a specific time. It simply grows for a while and then it stops dividing. But that can screw up some of your experiments. So, you do not want that to happen. So, if you want a given cell line, and which is not cancerous, but you want to keep them growing for a long time. So, there are ways in which you can immortalize a cell without making it cancerous. So, that cells those cells have to have a active telomerase activity.

The vast majority of cancer cells by default will have active telomerase activity. And in some stem cells, because stem cells also continue to divide. Stem cells when they are dividing, they always make a copy of themselves and a group of cells from this stem cell give rise to a tissue that has to originate from that stem cell. But it never make; it is just like you are taking money from your wallet, then it will empty stem cells also is like a reservoir.

So, if you keep on taking cells and differentiating, one fine day stem cell kitty will be empty. So, you do not want that to happen. So, what stem cells will do is just like you took 100 rupee from your wallet, you are making a copy of it and then putting back in the wallet and taking one. So, that there is no discount.

Making a copy here what I mean is not making Photostat of a currency that is what cell is capable of. You cannot make a copy of 100 rupee currency. But what if such a

situation was there so, your wallet will never empty. So, this logic you can maintain, you can follow, if these stem cells have the telomerase activity.

Otherwise, what will happen? Your newly formed stem cells as a part of cell division will be older and older and older. So, that will not be a friendly situation for cellular point of view or a cell differentiation point of view.

So, stem cells need to have this telomerase activity at least in some category. In other category what happens is these stem cells are capable of giving rise to the tissue only to a marginal extent. It is not stem cells forever. It is capable of giving rise to for a while and that is it, then it will not. Because it will exhaust or empty it is the stock of stem cells. So, such stem cells will not have the telomerase activity.

So, high telomerase activity exists in germ cells and the reason I told you because when a germ cell is being produces, say for example, sperm is being produced from a 18 year old fellow, 25 year old fellow, 35 year old fellow, 45 year old fellow.

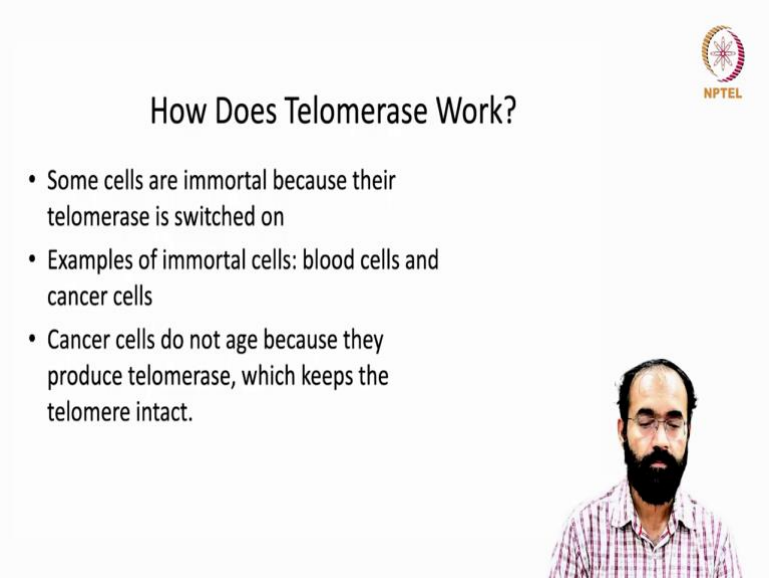
So, you cannot have variation in the quality of telomeres in this individual sperm, but the starting cell of a 18 year old fellow versus a 50 year old fellow the telomere length is significantly compromised. So, the telomerase have to compensate the length, so that the next generation is unaffected. So, germ cells must have to have adequate telomerase activity.

And epidermal skin cells, because your skin cells erode very fast. So, if they do not have telomerase, of course, telomerase activity comes down with old age. But their rate of division is so high that is why you know in cancer treatment, you know after chemotherapy people develop lots of hair loss, you know wrinkle of the skin, and they even have lot of vomiting, loose motion, etcetera, because the cancer cells divide very fast.

So, anti-cancer drugs are targeting all cells which divides, but your intestinal epithelial cells, skin cells, hair cells they divide faster than cancer cells. So, even before the cancer cells die, your healthy skin cells intestinal lining cells or hair cells they all will die, much before the cancer cells die, because they divide faster than cancer cells.

So, there are some cells in our body which divide faster than cancer cells, and they need to be maintained effectively. Hence, they also will have restricted telomerase activity, so that you will not start showing signs of old age. So, you have follicular hair cells and also cancer cells. So, cancer cells usually rejuvenate the telomerase. Normally, a given tissue a healthy tissue in your body do not have telomerase activity except these stem cells, epidermal skin cells like that. So, they rejuvenate and make them cancerous.

(Refer Slide Time: 13:32)



So, some cells are immortal because their telomerase is switched on. Because telomerase is an essential component or essential enzyme required for immortalization. Examples of immortal cells include the blood cells and the cancer cells. Blood cells why? Because your blood cell have got only 120 days lifespan so, the blood cell in your body, currently staying in your body will not be there after around 4 months, 4 month's time that cell will disappear the red blood cells.

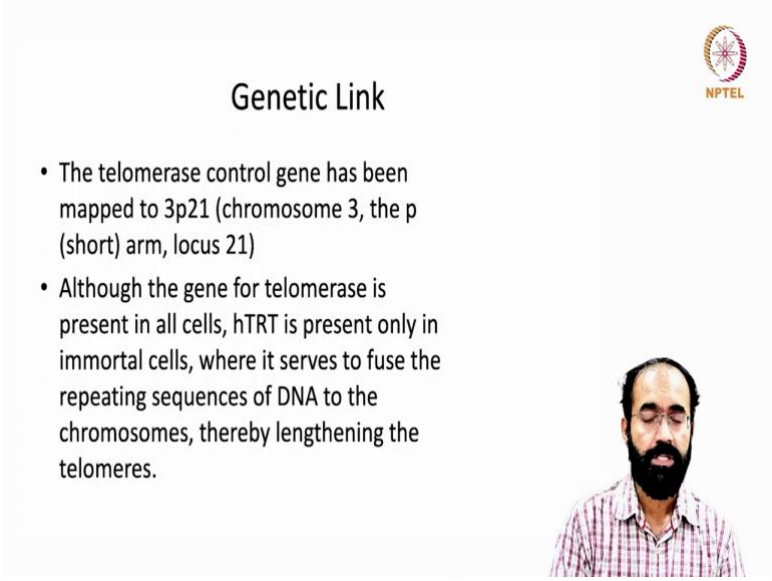
So, throughout your lifespan, 4 months is nothing, in like you need to have 3 full rounds of replication in 1 year. So, it is a lot. So, if a person living for say 50 years, you can imagine how many rounds these stem cells have to divide and in the bone marrow which produces this blood cells in the adult inside your bones. So, they have to divide and there are stem cells which give rise to the blood cells.

So, if they do not have this reviving ability of their telomeric ends, then immediately within no time, so maybe some 10 years or so, the organism will start showing signs of

senescence because the telomere length will decrease. So, you have bone marrow stem cells which is capable of producing your white blood cells and red blood cells which is seen in your a blood stream.

So, they need to have telomere length normalized. Because white blood cells only have the nucleus, normally, the RBCs do not have nucleus. In early stage of RBC formation, they have the nucleus. But in any case the bone marrow stem cells have the telomerase activity. So, cancer cells do not age because they produce telomerase which keeps the telomere length intact. So, the signs or the clues that direct a given cell into senescence or into apoptosis will be turned off in those situations.

(Refer Slide Time: 15:36)



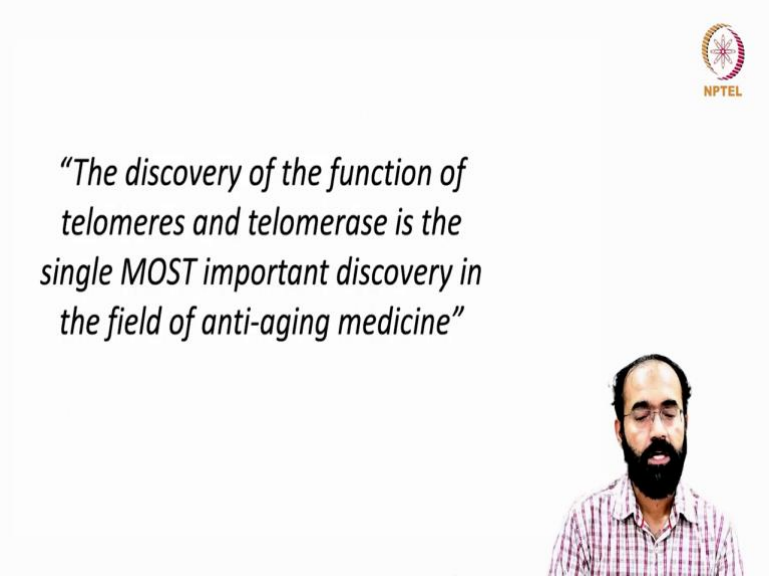
So, let us see what is the genetic link. The telomerase control gene has been mapped to 3p21, that is in the chromosome number 3, p arm, on locus 21 that is this notation indicate. Normally, every chromosome will have a p arm and a q arm; like p arm is the short arm, q arm is the longer arm.

So, which is on either side of the centromere. Centromere is a part in the chromosome during the cell division, the spindle fibres attach. It is just like a handle of your suitcase or your backpack. You have a handle. So, telomere is a handle for the spindle fibres to separate out this divided chromosomes. So, on either side of this centromere you have the p arm and the q arm. So, this is located on the p arm at the locus 21 this telomerase controlling gene.

Although, the gene for telomerase is present in all cells, human telomerase repeat transcript is present only in immortal cells. So, human telomeric specific reverse transcriptase hTRT. So, this telomeric repeat has to be added because of presence of a RNA template inside the telomerase enzyme.

Telomerase is a protein enzyme it has got an RNA template which is capable of producing this repeat. So, hTRT basically is the name of this enzyme. And it is usually seen only in the immortal cell, where it serves to fuse the repeating sequence of DNA to the chromosomes thereby lengthening the telomeres.

(Refer Slide Time: 17:26)

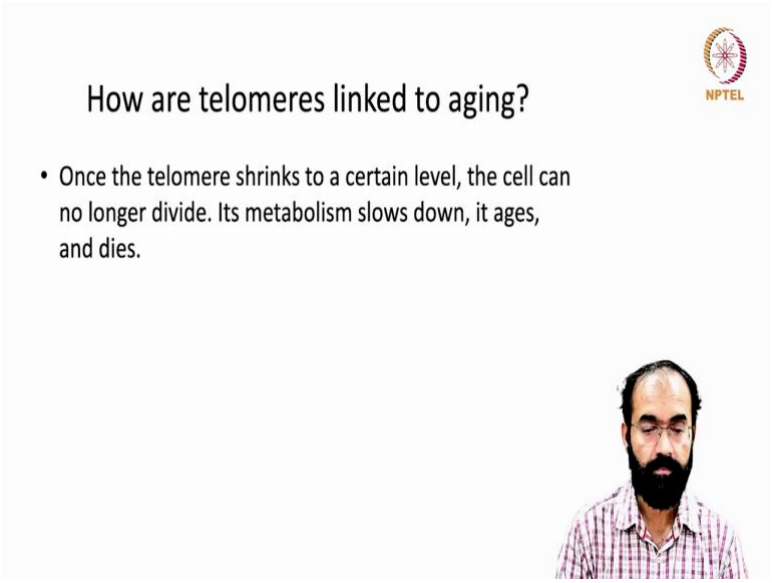


So, the discovery of function of telomeres and telomerase is the most important discovery in the field of anti-aging medicine. So, if we can tackle telomerase in a effective manner, there are many drugs available or many even plant derivatives, people are doing lots of research can we prolong the life of telomerase in a normal cell, so that you can prevent aging etcetera. Lot of research is going on in those lines.

(Refer Slide Time: 18:02)

Like people do lots of research on you know effect of applying turmeric. People, traditionally people apply turmeric on the skin to look young or some scars. So, the curcumin is the active ingredient which has got implications in cancer, lot of other you know cellular important events. So, people do a lot of study using this kind of you know naturally occurring compounds also, how telomere and telomerase stay in such a topical application of this kind of compounds.

(Refer Slide Time: 18:46)



So, let us see what is the role of telomeres and aging. How telomeres are linked to aging? So, aging we should understand is nothing to do with the calendar age. So, calendar age is something which nobody can stop. But what one can do is whether your body is reflecting the calendar age. Say for example, if a person is 40 year old in calendar, calendar age, 40 years passed since that person is born.

Now, the question is whether that person's body is also equal to 40 year or it is equal to 20 year or it is equal to 60 year. All these things are possible. So, 40 year is fixed one, it cannot change because that is the calendar year. Now, you are talking about the senescence or aging at a cellular point of view depends on how many rounds this particular person's body cells have divided.

Just like telephone or laptop batteries. People say your battery will stay healthy if you do charging and discharging cycles completely. I am not a battery expert, but people say you have to charge it and empty almost completely and again charge fully and again empty, completely. So, this is good for the battery life cycle. But sometimes people constantly keep the battery charged fully charged, that is also not good, people say, battery expert say.

So, in any case, the life of a battery is measured in terms of cycles, how many cycles of charging discharging cycle happen to that battery. Oh, a given battery can have around 1000 charge and discharge cycle, a typical lithium polymer battery. So, many a times if you have done this completely, then chances of your battery longevity is very high.

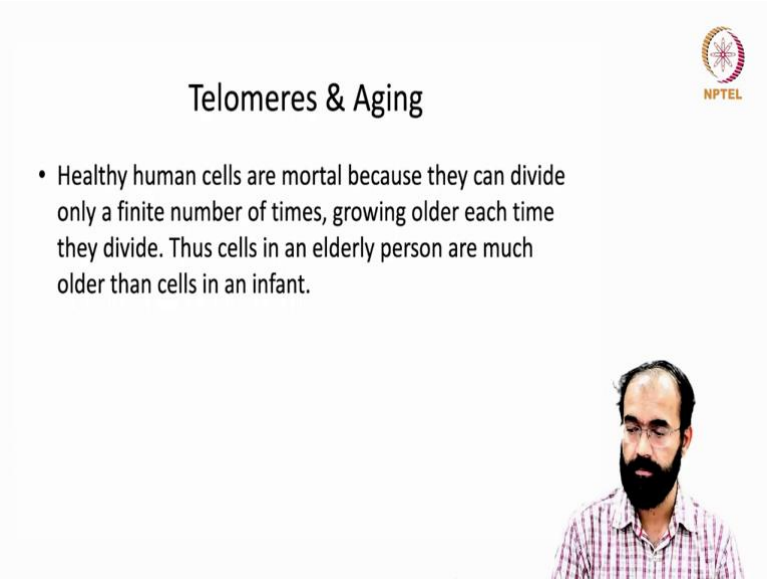
Same logic applies for your cell, if your cell a new cell is born in your body, say that cell is supposed to live for 3 months or 6 months, for convenience let us assume that it can live for 6 months. Of course, it varies from tissue to tissue. So, (Refer Time: 20:48) is on up to you, can I extend this 6 months to 1 year or will this 6 months longevity now going back to 3 months. So, if the longevity of that cell became 3 months because of your way you live the amount of toxins you push in into your body. Then what will happen?

When your 10 years passed the calendar age, instead of looking 10 year you are 20 years at a cellular level because 6 months age you shortened it to 3 months. Same way, the 6 months age you prolonged it to 12 months. Then what will happen? Once the 10 year have passed on calendar, you would be only 5 year would have passed in calendar. So,

cellular senescence and calendar senescence are something different which you should keep clearly in mind.

So, once the telomere shrinks to certain level, the cell can no longer divide because if it divides, it will have loss of gene function. So, its metabolism slows down and it ages and it dies, and this is normal situation that is called cellular senescence followed by apoptosis.

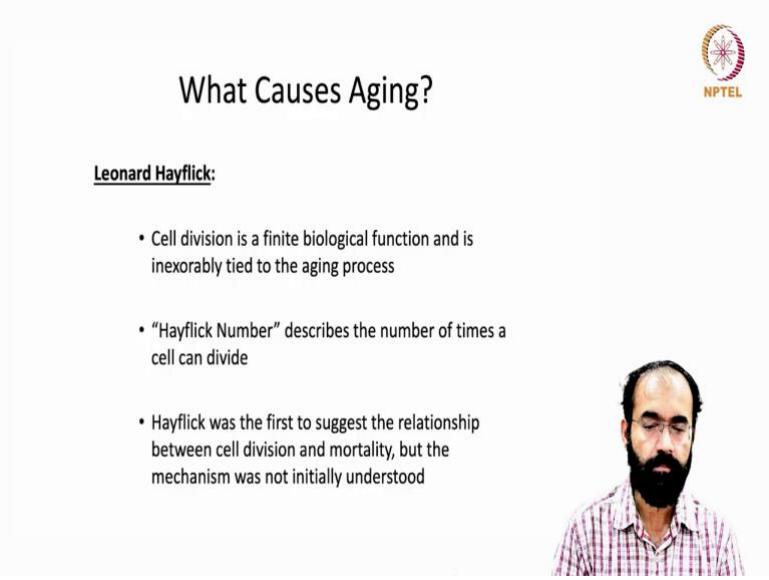
(Refer Slide Time: 21:53)



Healthy human cells are mortal, immortal is opposite of that. Mortal means it can die. And they are mortal because they can divide only a finite number of times, growing older each time when they divide. Thus, cells in an early elderly person are much older than the cells in an infant. And which you can clearly reflect also if you look at the skin. Skin is the easy part you can observe.

If you look at the skin of a young person versus an older person, you can clearly see it. And if some person, like some health cautious person might be maintaining their skin even younger than a infant, maybe because of the treatment. I am not talking about makeup, I am not talking about you know makeover or something, they are you know drinking adequate water, keeping their skin nourished etcetera. So, they are expanding or extending the lifespan of that cell and they look young.

(Refer Slide Time: 22:55)



So, cell division is a finite biological function and is inexorably tied to the aging process. Means you cannot separate these two. If a cell is dividing it will age. So, if today cells divided and became two cells tomorrow, they are older than the existing cell. It may sound little contradictory, means new cells are formed how can you say they are older than the pre-existing one.

Yes, a telomeric level or telomerase activity point of view or the length of the telomere which is say yardstick of future senescence, possible senescence. It is just like philosophically saying one day past means you reached one more day close to your death. It may sound little alarming, but that is a fact. You have a finite number of days of lifespan, whether you extend or do not extend it is up to you, your way of living.

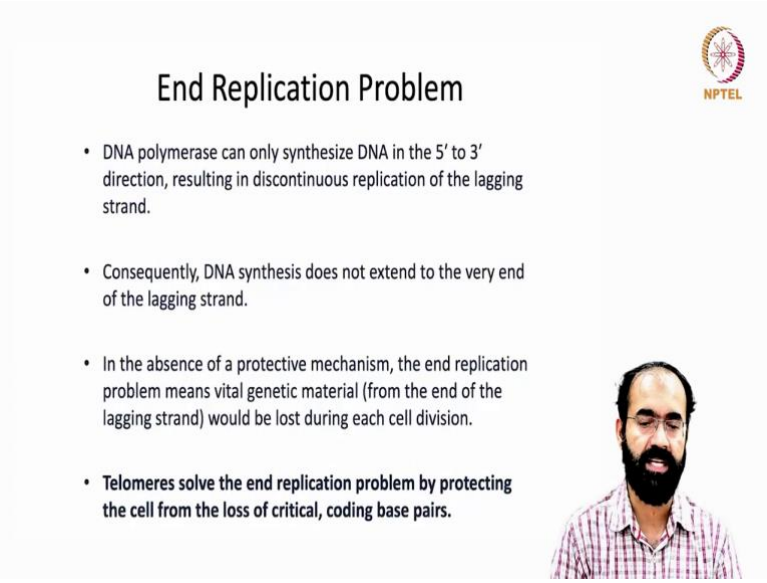
But it is normal that no one can say that, ok I will stay for 1 lakh years or 50,000 years, impossible. Maximum is 100 or around that ballpark figure. So, every day you are one step closer to your final day. So, same logic applies to every cell when they are dividing. The new cells are shorter. So, this concept should be clear to you that is why I am repeating. So, there is something called Hayflick number. You should be familiar with that. That describes the number of times a cell can divide.

So, Hayflick number can vary from which tissue you are talking about and the what is the length Hayflick number in a species also can vary based on. Like I gave the example of mice which shows the sign of senescence in F5; that means, they have a infinite

number of division capability, but that is not a permanent feature. So, Hayflick number describes the number of times a cell can divide.

Hayflick, Hayflick was the first scientist to suggest the relationship between cell division and mortality. So, but the mechanism was not initially understood. He was of the opinion that the more the cell divide, it is getting closer to death. So, this was the viewpoint which he was projecting.

(Refer Slide Time: 25:18)



So, later on people ended up finding out the end replication problem etcetera. Let us quickly go into the end replication problem. DNA polymerase can only synthesize DNA in the 5 prime to 3 prime direction, resulting in a discontinuous replication of DNA in the lagging strand, leading strand that is not an issue.

Consequently, what happens is DNA synthesis does not extend to the very end of the lagging strand. The reason I already told you. It cannot stand in the atmosphere or in the air and then synthesize the DNA. It need a template strand to hold clinch onto it, otherwise DNA polymerase will fall off from the DNA.

In the absence of a protective mechanism, the end replication problem means vital genetic material from the end of the lagging strand. Vital genetic material means not the telomeres, but the genes itself. It would be lost during each division. So, if a gene is supposed to be 1000 base pair, if the gene is the extreme end of the chromosome, 1000

base pair now will become say 990 base pair. So, 10 base is lost. So, you do not want that to happen. Then, the gene will start producing incomplete products.

So, telomeres solve the end replication problem by protecting the cell from the loss of critical coding base pairs. Telomeres do not have any coding sequence; hence it is perfectly fine. So, we will learn more about the telomeres and telomeres activity, and also Hayflick number in the next class.

Thank you.