

TELOMERASE: A POTENTIAL TARGET FOR DREADFUL DISEASES

**Vinayak M Gaware¹, Nachiket S Dighe¹, Shashikant R Pattan¹, Ravindra B Laware²,
Santosh B Dighe³, Deepak S Musmade¹, Mangesh B Hole¹**

1- Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy, Pravaranagar, 413736, (MS) India.

2- Department of Pharmaceutics, Pravara Rural College of Pharmacy, Pravaranagar, 413736, (MS) India.

3- Department of Pharmacology, Pravara Rural College of Pharmacy, Pravaranagar, 413736, (MS) India.

Summary

Telomerase, a eukaryotic ribonucleoprotein (RNP) complex, contains both an essential RNA and a protein reverse transcriptase subunit. The telomeres contain condensed DNA material, giving stability to the chromosomes. The enzyme is a reverse transcriptase that carries its own RNA molecule, which is used as a template when it elongates telomeres, which are shortened after each replication cycle. Over the past few years there has been significant progress in identifying the components of the telomerase holoenzyme complex and the proteins that associate with telomeres, in order to elucidate mechanisms of telomere length regulation. This review covers recent advances in the field including the use of telomerase in Cancer, ageing and heart diseases.

Keywords: Ageing, Cancer, Reverse Transcriptase, and Telomerase.

Address for Correspondence:

Mr. Nachiket S Dighe

Assistant Professor & HOD

Department of Pharmaceutical Chemistry, Pravara Rural College of Pharmacy,
Pravaranagar, A/P- Loni Bk. Taluka -Rahata, Dist-Ahmednagar 413736, India (MS).

E-mail-nachiket1111@rediffmail.com

Introduction

Telomerase is an enzyme that adds DNA sequence repeats ("TTAGGG" in all vertebrates) to the 3' end of DNA strands in the telomere regions, which are found at the ends of eukaryotic chromosomes. The telomeres contain condensed DNA material, giving stability to the chromosomes. The enzyme is a reverse transcriptase that carries its own RNA molecule, which is used as a template when it elongates telomeres, which are shortened after each replication cycle. The existence of a compensatory shortening of telomere (telomerase) mechanism, was first predicted by Soviet biologist Alexey Olovnikov in 1973¹ who also suggested the Telomere hypothesis of ageing and the Telomere relations to cancer. Telomerase was discovered by Carol W. Greider and Elizabeth Blackburn in 1985 in the ciliate *Tetrahymena*.² Together with Jack W. Szostak, Greider and Blackburn were awarded the 2009 Nobel Prize in Physiology or Medicine for their discovery. There are some indicators that telomerase is of retroviral origin.³

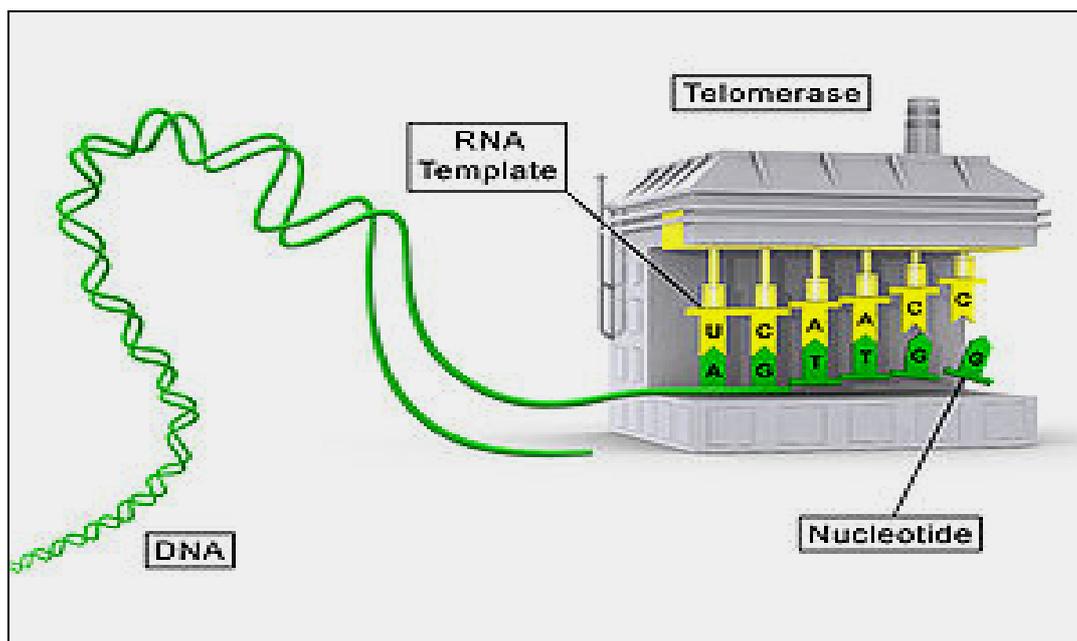


Figure 1: A conceptual diagram showing the protein component of telomerase (TERT) in grey and the RNA component (TR) in yellow.

History ⁴

1984	Blackburn was propelled to prominence in the cancer field after she co-discovered with Carol Greider the enzyme telomerase.
1985	In 1985, while a professor at University of California, Berkeley, Blackburn and her then-graduate student Greider reported the discovery of telomerase. Their research showed that, in some organisms, such as the single-celled pond dweller Tetrahymena, telomerase
1994	Telomerase is unique as an immortalizing agent," Dr. Harley said in an interview at Geron's headquarters. Although today's publication deals specifically with ovarian cancer, Dr. Harley said his collaborators had found that "telomerase is found in all cancer cells studied.
1997	Since the discovery of telomerase, its presence in tumor cells has raised hopes that it may offer a pre-eminent target for anticancer drugs. "The cloning of the active center of telomerase is a major milestone that sets the stage for more fully understanding the molecular genetics
1998	The Geron Corporation of Menlo Park, Calif., which owns or has applied for several patents on the gene, known as the telomerase gene. Experts cautioned that as important as the new results were, there was a Catch-22 in the way evolution had designed the telomerase system.
1999	Normal human cells don't have active telomerase, but they have the blueprint to make it," said William Hahn, a postdoctoral fellow in oncology at ... Mice have telomerase active in their cells all the time and it turns out that's what makes it easy to induce cancer in the rodents.
2003	Experts know that a molecule called telomerase, which is switched off in normal cells, is one of the secrets behind cancer's eternal youth. Lead researcher Professor Robert Newbold, from Brunel University in Uxbridge, said: "Telomerase is crucial in allowing our cells to keep on.
2004	The longer a woman had been caring for a sick child, the shorter her telomeres, the lower her levels of telomerase and the higher her levels of "oxidative. The researchers also measured levels of an enzyme called telomerase, which helps rebuild telomeres to stave off this process.
2005	"In ancient Egypt, men smeared their pates with hippopotamus fat in a desperate bid to stave off baldness," she said. "Is telomerase the new hippopotamus fat?"
2009	Three Americans won the Nobel prize for medicine on Monday for revealing the existence and nature of telomerase. The trio's work laid the foundation for understanding how telomerase and telomeres. the small caps on the end of chromosomes that carry the DNA.

Table 1: History of Telomerase

Structure

The protein composition of human telomerase, identified in 2007 by Scott Cohen and his team at the Children's Medical Research Institute in Australia. It consists of two molecules each of human Telomerase Reverse Transcriptase (TERT), Telomerase RNA (TR or TERC) and dyskerin (DKC1). The genes of telomerase subunits, which are TERT, TERC, DKC1 and TEP1 *etc*, are located on the different chromosomes in human genome. Human TERT gene(hTERT) is translated into a protein of 1132 amino acids.⁴ TERT proteins are sequenced in many eukaryotes. TERT polypeptide folds with TERC, a non-coding RNA (451 nucleotides long in human). TERT has a 'mitten' structure that allows it to wrap around the chromosome to add single-stranded telomere repeats. TERT is a reverse transcriptase, which is a class of enzyme that creates single-stranded DNA using single-stranded RNA as a template.⁵ Enzymes of this class (not TERT specifically, but the ones isolated from viruses) are utilized by scientists in the molecular biological process of Reverse Transcriptase PCR (RT-PCR), which allows the creation of several DNA copies of a target sequence using RNA as a template. As stated above, TERT carries its own template around, TERC. The high resolution protein structure of the *Tribolium castaneum* catalytic subunit of telomerase TERT was decoded in 2008 by Emmanuel Skordalakes and his team at The Wistar Institute in Philadelphia. The structure revealed that the protein consists of four conserved domains (RNA-Binding Domain (TRBD), fingers, palm and thumb), organized into a ring configuration that shares common features with retroviral reverse transcriptases, viral RNA polymerases and bacteriophage B-family DNA polymerases.⁶

Function

By using TERC, TERT can add a six-nucleotide repeating sequence, 5'-TTAGGG (in all vertebrates, the sequence differs in other organisms) to the 3' strand of chromosomes. These TTAGGG repeats (with their various protein binding partners) are called telomeres.⁷ The template region of TERC is 3'-CAAUCCCAAUC-5'. This way, telomerase can bind the first few nucleotides of the template to the last telomere sequence on the chromosome, add a new telomere repeat (5'-GGTTAG-3') sequence, let go, realign the new 3'-end of telomere to the template and repeat the process.⁸

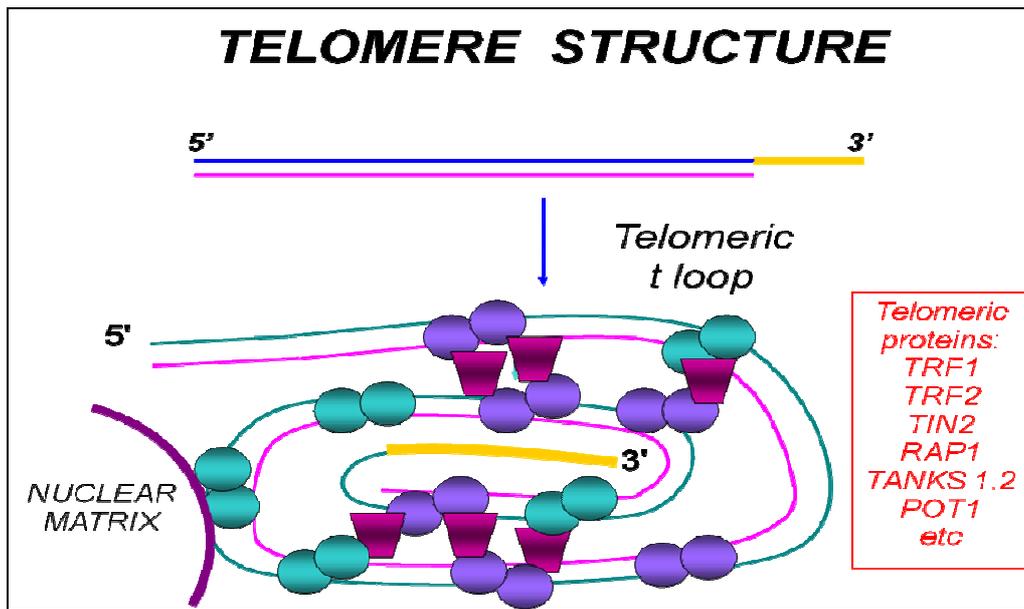


Figure 2: Structure of Telomere

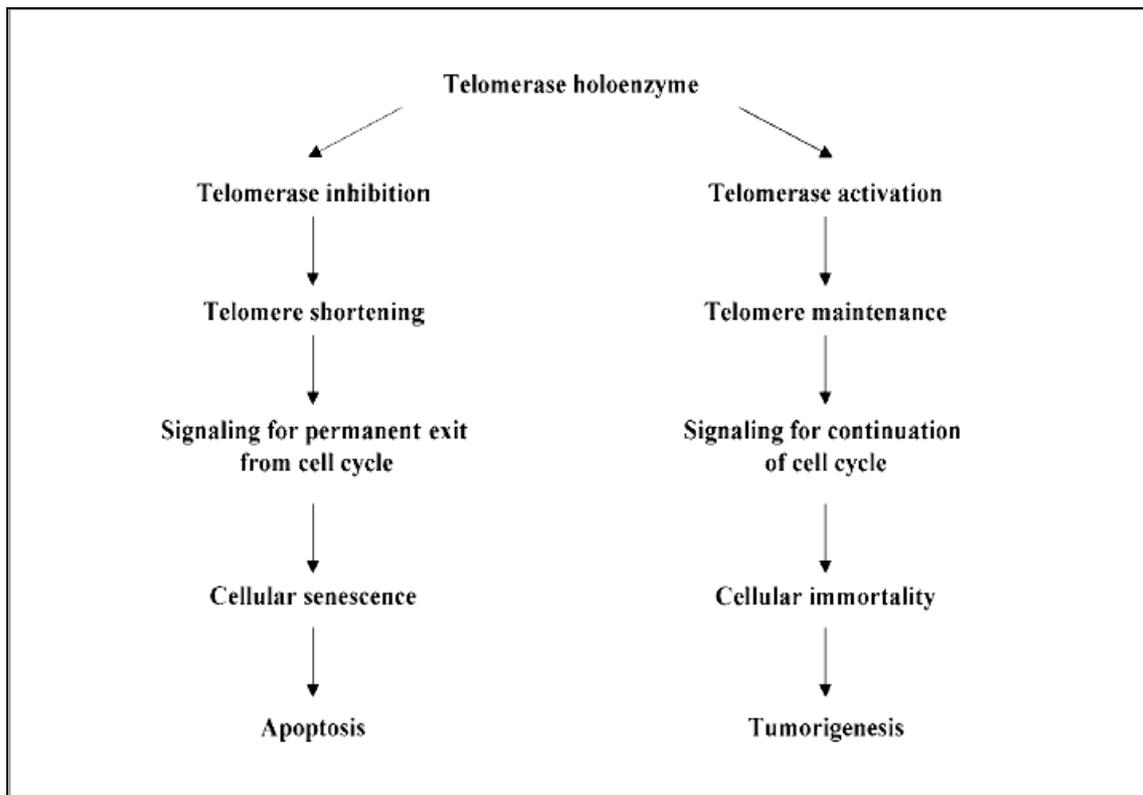


Figure 3: Function of Telomerase

Telomerase reverse transcriptase

Telomerase reverse transcriptase (abbreviated to **TERT** or **hTERT** in humans) is a catalytic subunit of the enzyme telomerase. Its absence (usually as a result of a chromosomal mutation) is associated with the disorder Cri du chat.⁹ Telomerase is a ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG. The enzyme consists of a protein component with reverse transcriptase activity, encoded by this gene and an RNA component which serves as a template for the telomere repeat. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis.¹⁰ Studies in mice suggest that telomerase also participates in chromosomal repair, since de novo synthesis of telomere repeats may occur at double-stranded breaks. Alternatively spliced variants encoding different isoforms of telomerase reverse transcriptase have been identified; the full-length sequence of some variants has not been determined. Alternative splicing at this locus is thought to be one mechanism of regulation of telomerase activity.¹¹

Mechanism of action of telomerase

This ribonucleoprotein complex elongates the 3' telomeric end of the lagging-strand DNA template by a reiterative reverse transcription mechanism. The action of the telomerase from *Oxytricha*, which adds a T₄G₄ repeat unit, is depicted; other telomerases add slightly different sequences. The telomerase contains an RNA template (red) that base-pairs to the 3' end of the lagging-strand template.¹² The telomerase catalytic site (green) then adds deoxyribonucleotides (blue) using the RNA molecule as a template; this reverse transcription proceeds to position 35 of the RNA template (step 1).¹³ The strands of the resulting DNA-RNA duplex are then thought to slip relative to one another, leading to displacement of a single-stranded region of the telomeric DNA strand and to uncovering of part of the RNA template sequence (step 2). The lagging-strand telomeric sequence is again extended to position 35 by telomerase and the DNA-RNA duplex undergoes translocation and hybridization as before (steps 3 and 4).¹⁴

The slippage mechanism is thought to be facilitated by the unusual base pairing (black dots) between the displaced G residues, which is less stable than Watson-Crick base pairing. Telomerase can add very long stretches of repeats by repetition of steps (4) and (5).¹⁵

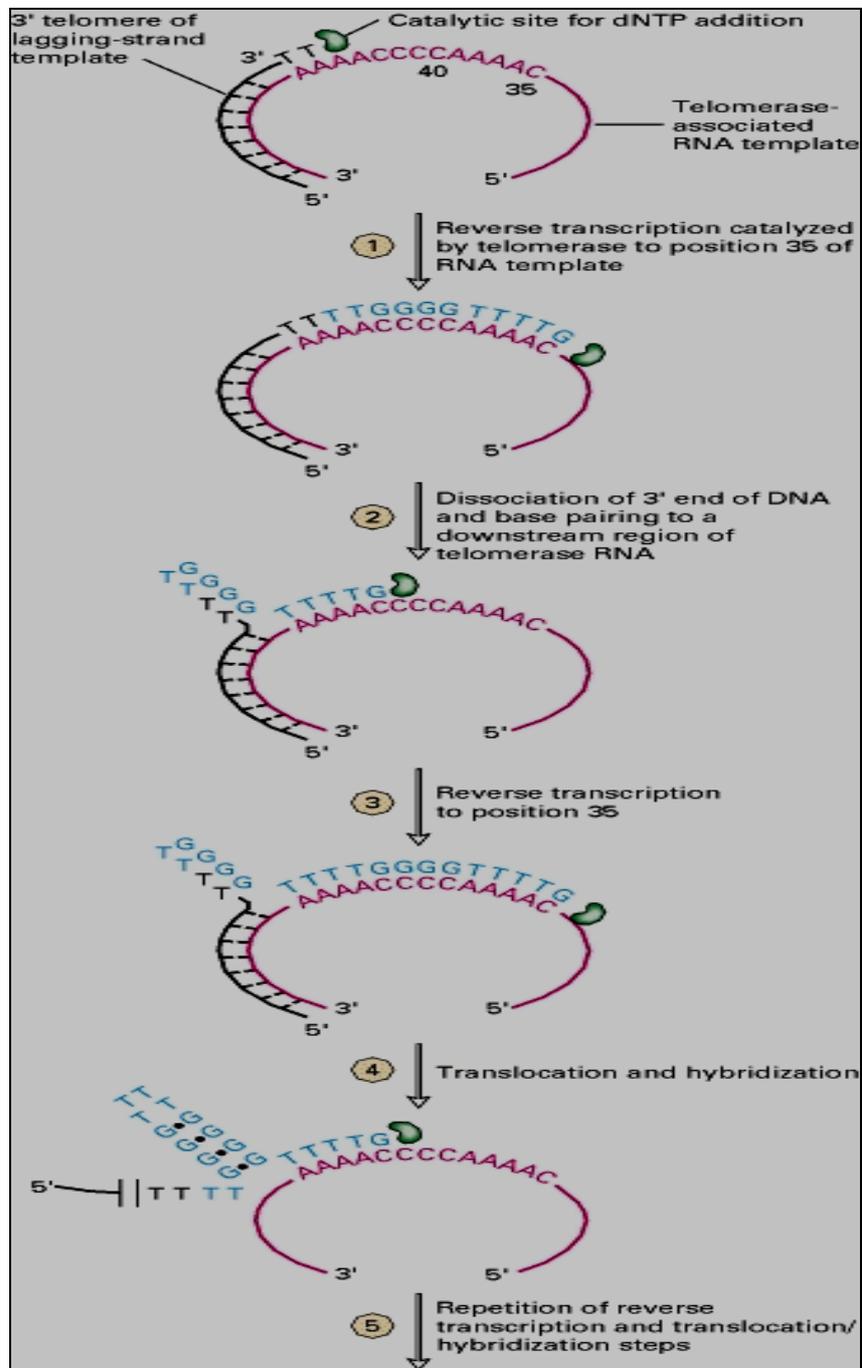


Figure 4: Mechanism of action of telomerase

Methods Used in Evaluating Telomerase Activity^{16, 17, 18}

- Original TRAP assay
- Fluorescent-TRAP
- Stretch-PCR
- Stretch-PCR and Pico Green
- In situ TRAP
- TMA/HPA
- RT-PCR
- Real-time PCR
- Telomerase in intact nuclei

Clinical implications

Telomerase and Aging

Synthesis of the lagging strand requires a short primer, which will be removed. At the extreme end of a chromosome, there is no way to synthesize this region when the last primer is removed. Therefore, the lagging strand is always shorter than its template by at least the length of the primer. This is the so-called "**end-replication problem**".¹⁹ Bacteria do not have the end-replication problem, because its DNA is circular. In eukaryotes, the chromosome ends are called telomeres which have at least two functions:

- To protect chromosomes from fusing with each other.
- To solve the end-replication problem.

The procedure to solve the end-replication problem is outlined in **Figure 5**. Mechanism of the telomere extension by telomerase is explained in **Figure 6**.¹⁹

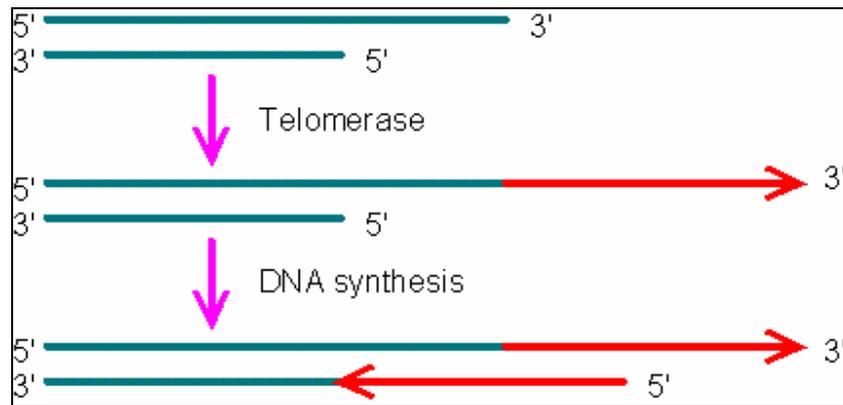


Figure 5: Telomerase and telomere extension. To extend the length of a telomere, the telomerase first extends its longer strand. Then, using the same mechanism as synthesizing the lagging strand, the shorter strand is extended.

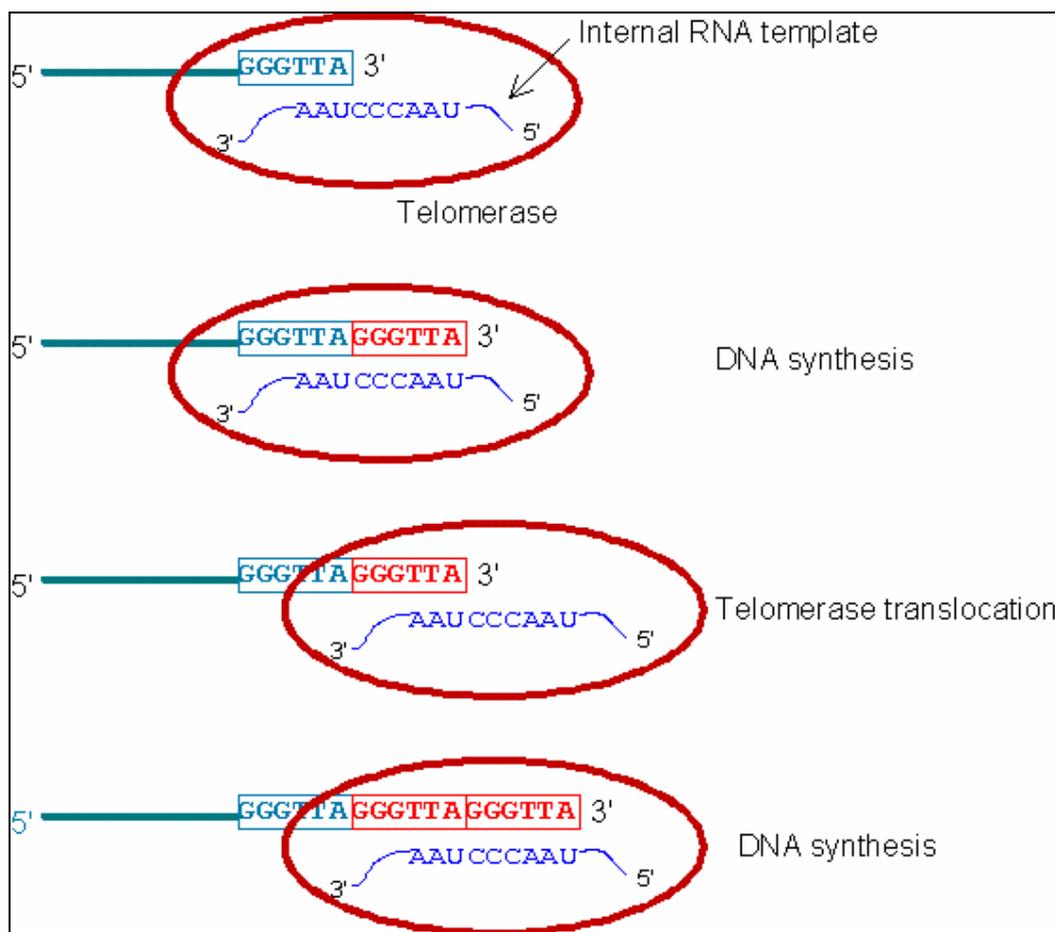


Figure 6: The mechanism of telomere extension by telomerase.

In a human chromosome, the telomere is about 10 to 15 kb in length, composed of the tandem repeat sequence: TTAGGG.²¹ The telomerase contains an essential RNA component which is complementary to the telomere repeat sequence. Hence, the internal RNA can serve as the template for synthesizing DNA. Through telomerase translocation, a telomere may be extended by many repeats.²²

Aging

In the absence of telomerase, the telomere will become shorter after each cell division. When it reaches a certain length, the cell may cease to divide and die. Therefore, telomerase plays a critical role in the aging process.²³

Cancer

When cells are approaching the Hayflick limit in cell cultures, the time to senescence can be extended by the inactivation of the tumor suppressor proteins - TP53 and Retinoblastoma protein (pRb). Cells that have been so-altered will eventually undergo an event termed a "crisis" when the majority of the cells in the culture die. Sometimes, a cell does not stop dividing once it reaches crisis. In a typical situation, the telomeres are lost and the integrity of the chromosomes declines with every subsequent cell division. Exposed chromosome ends are interpreted as double-stranded breaks (DSB) in DNA; such damage is usually repaired by reattaching (religating) the broken ends together. When the cell does this due to telomere-shortening, the ends of different chromosomes can be attached together. This temporarily solves the problem of lacking telomeres; but, during anaphase of cell division, the fused chromosomes are randomly ripped apart, causing many mutations and chromosomal abnormalities. As this process continues, the cell's genome becomes unstable. Eventually, either sufficient damage will be done to the cell's chromosomes such that cell dies (via programmed cell death, apoptosis), or an additional mutation that activates telomerase will take place. With the activation of telomerase, some types of cells and their offspring become immortal, that is, their chromosomes will not become unstable no matter how many cell divisions they undergo (they bypass the Hayflick limit), thus avoiding cell death as long as the conditions for their duplication are met. Many cancer cells are

considered 'immortal' because telomerase activity allows them to divide virtually forever, which is why they can form tumors. A good example of cancer cells' immortality is HeLa cells, which have been used in laboratories as a model cell line since 1951. They are indeed immortal - daily production of HeLa cells is estimated at several tons even up to this day. While this method of modeling human cancer in cell culture is effective and has been used for many years by scientists, it is also very imprecise. The exact changes that allow for the formation of the tumorigenic clones in the above-described experiment are not clear. Scientists have subsequently been able to address this question by the serial introduction of several mutations present in a variety of human cancers.²⁴ This has led to the elucidation of several combinations of mutations that are sufficient for the formation of tumorigenic cells, in a variety of cell types. While the combination varies depending on the cell type, a common theme is that the following alterations are required: activation of TERT, loss of p53 pathway function, loss of pRb pathway function, activation of the Ras or myc proto-oncogenes and aberration of the PP2A protein phosphatase. That is to say, the cell has an activated telomerase, eliminating the process of death by chromosome instability or loss, absence of apoptosis-induction pathways and continued activation of mitosis. This model of cancer in cell culture accurately describes the role of telomerase in actual human tumors. Telomerase activation has been observed in ~90% of all human tumors, suggesting that the immortality conferred by telomerase plays a key role in cancer development. Of the tumors that have not activated TERT, most have found a separate pathway to maintain telomere length termed ALT (Alternative Lengthening of Telomeres). The exact mechanism behind telomere maintenance in the ALT pathway has not been elucidated, but likely involves multiple recombination events at the telomere.²⁵

Additional roles in cancer, heart disease and a socioeconomic and quality of life aspect

Additional roles for telomerase per work include the upregulation of 70 genes known or suspected in cancers' growth and spread through the body and the activation of glycolysis, which enables cancer cells to rapidly use sugar to facilitate their programmed growth rate. (roughly the growth rate of a fetus) (MIT) recently imaged colon cancer stem cells and compared them to fetal colon stem cells trying to make a new colon; they were the same. UCSF has shown work that reveals that mothers caring for their very sick children have shorter telomeres when they report that their emotional stress is at the greatest point. She also found telomerase active at the

site of blockages in coronary artery tissue. This could be why heart attacks can come on so suddenly: Telomerase is driving the growth of the blockage. Other work has shown that the poor of society have shorter telomeres than the rich. Short telomeres can lead to telomeric crisis and the initiation of cancer if many other conditions are also met, or so the discussion goes at this point. Blackburn and the two other co-discoverers of telomerase won the Lasker Award (2006) and the Nobel Prize (2009) for the discovery of telomerase and subsequent work on telomerase. Blackburn also won the 2006 Gruber Genetics Prize for same.²⁶

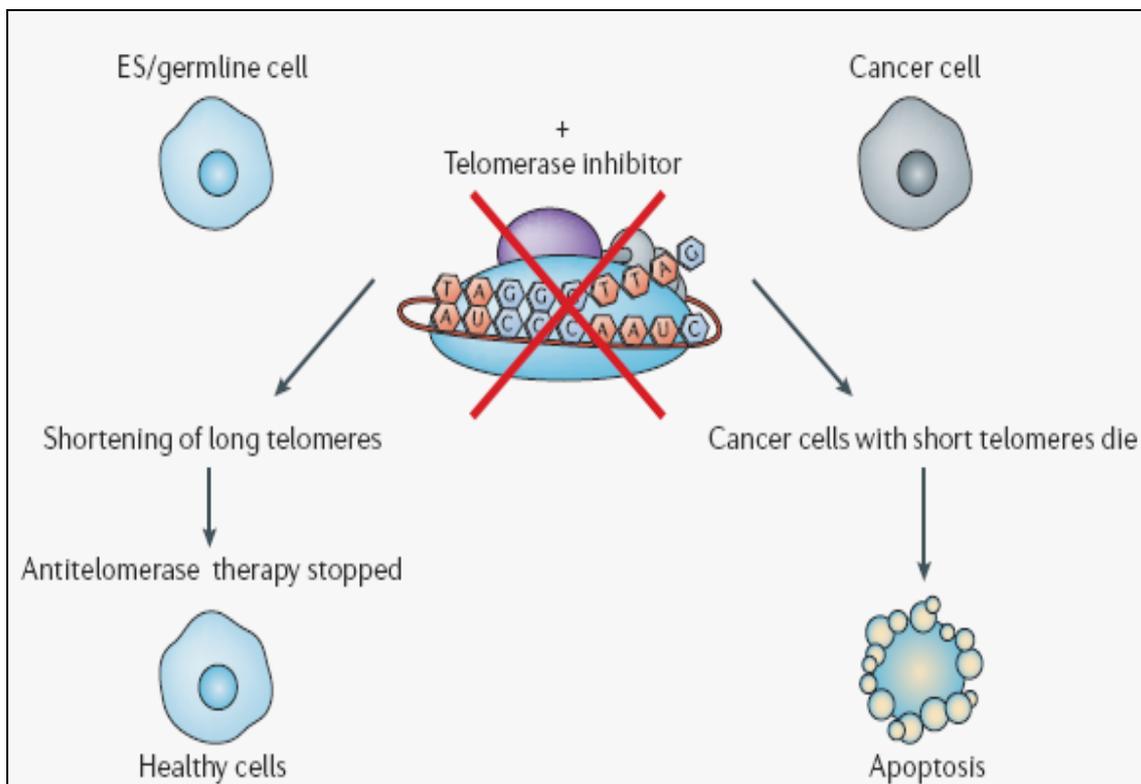


Figure 7: Comparing telomerase inhibition in normal versus cancer cells.

Role in other human diseases

Mutations in TERT have been implicated in predisposing patients to aplastic anemia, a disorder in which the bone marrow fails to produce blood cells, in 2005. Cri du chat Syndrome (CdCS) is a complex disorder involving the loss of the distal portion of the short arm of chromosome 5.

TERT is located in the deleted region and loss of one copy of TERT has been suggested as a cause or contributing factor of this disease. Dyskeratosis congenita (DC) is a disease of the bone marrow that can be caused by some mutations in the telomerase subunits. In the DC cases, about 35% cases are X-linked-recessive on the DKC1 locus and 5% cases are autosomal dominant on the TERT and TERC loci. Patients with DC have severe bone marrow failure manifesting as abnormal skin pigmentation, leucoplakia (a white thickening of the oral mucosa) and nail dystrophy, as well as a variety of other symptoms. Individuals with either TERC or DKC1 mutations have shorter telomeres and defective telomerase activity *in vitro* than other individuals of the same age. There has also been one family in which autosomal dominant DC has been linked to a heterozygous mutation in TERT. These patients also exhibited an increased rate of telomere-shortening and genetic anticipation (i.e., the DC phenotype worsened with each generation).²⁷

Telomerase as a potential drug target

Cancer is a very difficult disease to fight because the immune system has trouble recognizing it and cancer cells are immortal; they will always continue dividing. Because telomerase is necessary for the immortality of so many cancer types, it is thought to be a potential drug target. If a drug can be used to turn off telomerase in cancer cells, the above process of telomere-shortening will resume telomere length will be lost as the cells continue to divide, mutations will occur and cell stability will decrease. Experimental drug and vaccine therapies targeting active telomerase have been tested in mouse models and some have now entered early clinical trials. Geron Corporation is currently conducting four human clinical trials involving telomerase inhibition and telomerase vaccination. Merck, as a licensee of Geron, has recent approval of an IND for one vaccine type. The vaccine platform is being tested (and now jointly with Merck) using three different approaches. One vaccine is adenovirus/plasmid based (Merck IND). The second is an autologous dendritic cell based vaccine (GRNVAC1), formerly called TVAX when tested in Phase I clinical trials in Prostate Cancer and it showed significant PSA doubling times as well as T-cell response. Geron's embryonic stem cell derived dendritic cell vaccine targeting telomerase is the third approach and is currently at the pre-clinical stage. These vaccine methods attempt to teach the human immune system to attack cancer cells expressing telomerase. Geron's telomerase inhibitor drug (GRN163L) attempts to stop cancer cell proliferation by inhibiting

telomerase and it is in three separate early stage human clinical trials. Indeed, telomerase inhibition in many types of cancer cells grown in culture has led to the massive death of the cell population. However, a variety of caveats, including the presence of the ALT pathway,^{[22][23]} complicate such therapies.²⁷ Some have reported ALT methods of telomere maintenance and storage of DNA in cancer stem cells, however Geron claims to have killed cancer stem cells with their telomerase inhibitor GRN163L at Johns Hopkins. GRN163L binds directly to the RNA template of telomerase. Even a mutation of the RNA template of telomerase would render the telomerase unable to extend telomeres and therefore not be able to grant replicative immortality to cancer, not allow glycolysis to be initiated and not upregulate Blackburn's 70 cancer genes. Since Blackburn has shown that most of the harmful cancer-related effects of telomerase are dependent on an intact RNA template, it seems a very worthwhile target for drug development. If indeed some cancer stem cells use an alternative method of telomere maintenance, it should be noted that they are still killed when the RNA template of telomerase is blocked. According to Blackburn's opinion at most of her lectures, it is a big mistake to think that telomerase is involved with only extending telomeres. Stopping glycolysis in cancer stem cells and preventing the upregulation of 70 bad genes is probably what is killing cancer stem cells if they are using alternative methods.²⁸

Telomerase as a diagnostic tool²⁹

- Could be used as a marker for cancer diagnostics, prognosis, patient monitoring and screening
- Telomerase activity indicative of cancer cells

Side effects include³⁰

- **Blood toxicity:** Some populations of stem cells, which are the parents of mature blood cells, do use telomerase. Anti-telomerase drugs could, therefore, suppress the production of vital blood cells.
- **Immune toxicity:** Some infection-fighting cells use telomerase normally. Anti-telomerase drugs, therefore, could theoretically weaken our ability to fight infection.

- **Skin toxicity:** While most of our skin cells have little telomerase activity, those that repair wounds do have some. Anti-telomerase drugs might cause delayed wound healing.
- **Gonadal toxicity:** Some normal telomerase activity is seen in the cells of the ovary and testis. Thus, anti-telomerase drugs could potentially interfere with fertility, although this is still speculative.

One drawback to the use of anti-telomerase drugs in the treatment of cancer is the length of time needed for such drugs to have any effects. If telomerase is not activated until numerous generations of cell division have shortened telomeres to critical lengths, tumor cells could have doubled thirty or more times before telomerase is turned on. Even if anti-telomerase drugs were developed in the near future, they would need to be used in conjunction with faster-acting anti-cancer drugs.

The future research³⁰

- **Cells from diseased tissue can be telomerase-immortalized**
 - Function comparably well to non-immortalized counterparts
 - Explore mechanism of disease
 - Develop interventions for treatment and prevention
- Wound healing
- Tissue regeneration (ex: burn victims)
 - Problem: How do you stop treated cells from becoming cancerous?
- Age related diseases
 - Atherosclerosis, macular degeneration (eye)
- Take patient's cells, manipulate and rejuvenate them, then reinsert them into their body
 - Expansion of specific immune cells or nerve cell precursors
 - Possible treatments
 - Immune deficiencies or neurodegenerative diseases
- Continued cancer research
 - Peptide Epithalon and how it induces telomerase activity

Conclusion

There have been many recent significant developments in the telomere/telomerase fields of research, but there are still many gaps in our understanding. More preclinical proof-of-efficacy studies and additional clinical trials are required. The progress made in the past 2 years has been impressive and there is an emerging general consensus that telomerase-targeted therapies are a promising and novel approach to cancer therapeutics that could lead to effective interventions for the treatment of cancer with minimal side effects. Although one can always make arguments for and against any novel cancer therapeutic, the preclinical and emerging clinical experimental evidence for telomerase as a relatively universal target for cancer therapy is encouraging, and targeting telomere-maintenance mechanisms continues to be an exciting prospect in our repertoire of future cancer strategies. Importantly, we need to establish how ageing (and cellular replicative senescence) contribute to actual human physiology and how its dysregulation can contribute to cancer progression.

References

1. Lovnikov AM. A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. *J Theor Biol.* 1973 14;41(1):181-90.
2. Greider, C.W. & Blackburn, E.H. "Identification of a specific telomere terminal transferase activity in Tetrahymena extracts". *Cell*, 1985, 43 : 405–413.
3. Witzany, G. "The Viral Origins of Telomeres and Telomerases and their Important Role in Eukaryogenesis and Genome Maintenance". *Biosemiotics* 2008, 1: 191–206.
4. Watson JD. Origin of concatemeric T7 DNA. *Nat New Biol*, 1972;239: 197-201.
5. Cohen S, Graham M, Lovrecz G, Bache N, Robinson P, Reddel R "Protein composition of catalytically active human telomerase from immortal cells". *Science* 2007, 315 (5820): 1850–3.
6. Gillis, A. J.; Schuller, A. P. & Skordalakes, E. "Structure of the *Tribolium castaneum* telomerase catalytic subunit TERT", *Nature*, 2008, 455 (7213): 633–637.
7. Gavory G, Farrow M, Balasubramanian S, "Minimum length requirement of the alignment domain of human telomerase RNA to sustain catalytic activity in vitro". *Nucleic Acids Res.* 2002, 30 (20): 4470–80.
8. Hayflick L, Moorhead PS. "The serial cultivation of human diploid cell strains". *Exp Cell Res* 1961, 25: 585–621.
9. Blasco MA. Telomeres and human disease: aging, cancer and beyond. *Nat Rev Genet.* 2005 Aug;6(8):611-22.
10. de Magalhaes JP, Toussain O. Telomeres and telomerase: a modern fountain of youth? *Rejuvenation Res.* 2004;7(2):126-33.
11. Tomás-Loba A, Flores I, Fernández-Marcos PJ, Cayuela ML, Maraver A, Tejera A, Borrás C, Matheu A, Klatt P, Flores JM, Viña J, Serrano M, Blasco MA. Telomerase reverse transcriptase delays aging in cancer-resistant mice. *Cell.* 2008 14;135(4):609-22.
12. Tanglao, Shanna, et al. The Discovery of C0057684, a Telomerase Activity Inducing Compound. The Methuselah Foundation. 2008, 11: 157-160.
13. Sierra Sciences. Cure Aging or Die Trying, 2009, 4: 123-126.
14. Britt, Robert Roy, One Key Found for Living to 100. *LiveScience.* 2009, 12: 222- 227.

15. Bryan TM, Englezou A, Gupta J, Bacchetti S, Reddel RR. Telomere elongation in immortal human cells without detectable telomerase activity. *EMBO J.* 1995 1;14(17):4240-8.
16. Henson JD, Neumann AA, Yeager TR, Reddel RR "Alternative lengthening of telomeres in mammalian cells". *Oncogene*, 2002, 21 (4): 598–610.
17. Yamaguchi, H.; Calado, R. T.; Ly, H.; Kajigaya, S.; Baerlocher, G. M.; Chanoock, S. J.; Lansdorp, P. M. & Young, N. S., "Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia", *New England Journal of Medicine*, 2005, 352 (14): 123.
18. Zhang, A.; Zheng, C.; Hou, M.; Lindvall, C.; Li, K. J.; Erlandsson, F.; Bjorkholm, M.; Gruber, A. et al. "Deletion of the telomerase reverse transcriptase gene and haploinsufficiency of telomere maintenance in Cri du chat syndrome", *The American Journal of Human Genetics*, 2003, 72 (4): 940–948.
19. Yamaguchi H "Mutations of telomerase complex genes linked to bone marrow failures" *J Nippon Med Sch.* 2007, 74 (3): 202–9.
20. Heiss NS, Knight SW, Vulliamy TJ et al. "X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions". *Nat Genet.* 1998, 19 (1): 32–8.
21. Vulliamy TJ, Walne A, Baskaradas A et al.. "Mutations in the reverse transcriptase component of telomerase (TERT) in patients with bone marrow failure". *Blood Cells Mol Dis.* 2005, 34 (3): 257–63.
22. Vulliamy T, Marrone A, Goldman F et al."The RNA component of telomerase is mutated in autosomal dominant dyskeratosis congenita". *Nature*, 2001, 413 (6854): 432–5.
23. Marrone, A.; Walne, A. & Dokal, I. , "Dyskeratosis congenita: telomerase, telomeres and anticipation", *Current Opinion in Genetics & Development* 2005, 15 (3): 249–257.
24. Armanios, M.; Chen, J. L.; Chang, Y. P.; Brodsky, R. A.; Hawkins, A.; Griffin, C. A.; Eshleman, J. R.; Cohen, A. R. et al., "Haploinsufficiency of telomerase reverse transcriptase leads to anticipation in autosomal dominant dyskeratosis congenita", *PNAS* 2005, 102 (44): 15960–15964.
25. Kirkpatrick KL, Mokbel K, "The significance of human telomerase reverse transcriptase (hTERT) in cancer". *Eur J Surg Oncol*, 2001, 27 (8): 754–60.
26. Zhang A, Zheng C, Hou M, Lindvall C, Li KJ, Erlandsson F, Björkholm M, Gruber A, Blennow E, Xu D, "Deletion of the telomerase reverse transcriptase gene and haploinsufficiency of telomere maintenance in Cri du chat syndrome". *Am. J. Hum. Genet.* 2003, 72 (4): 940–8.
27. Cerruti Mainardi P. "Cri du Chat syndrome". *Orphanet J Rare Dis*, 2006, 1: 33.
28. Altshuler, M.L., S.E. Severin and A.I. Glukhov. "The Tumor Cell and Telomerase." *Biochemistry (Moscow)*. , 2003, 68 (12):1275-1283.
29. Clark, William R. *A Means to an End: The Biological Basis of Aging and Death.* Oxford University Press, New York. 1999, 10: 445-447.
30. Colangelo, D., A. L. Ghiglia, I. Viano, G. Cavigliolo and D. Osella. "Cis Pt(II)(pyridine)(5-SO₃H-isoquinoline)] complex, a selective inhibitor of telomerase enzyme." *BioMetals*, 2003, 16: 553-560.
31. Li, H. and J-P Liu. "Signaling on telomerase: a master switch in cell aging and immortalization." *Biogerontology*, 2002, 3: 107-116.