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Comment

hTERT Promoter Regulation by Differentiation Mechanisms vs Telomerase Activity in Somatic, Embryonic, and Cancerous Cells

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Abstract

Telomere shortening in the somatic cells is one of the most well-documented factors of cellular ageing. Telomeres are composed of tandem hexanucleotide repeats that protect cells from unwanted recombination mechanisms, secure the ends of chromosomes and their stability, and are responsible for limited division capacity. Telomerase is an enzymatic ribonucleoprotein complex, present in embryonic cells, adult stem cells, and germinal progenitors, whose function is to extend the telomeres length by adding the lost tandem repeats. The main component of the complex and its rate-limiting agent is reverse transcriptase (in humans, hTERT). It has been shown in multiple studies that the differentiated state of the cell corresponds to the cell's telomerase activity and vice versa. This article discusses a proposal which claims that a strong biomolecular correlation between differentiation factors and the hTERT regulation exists. If to discover what exact mechanisms stay behind this relatedness, the fields of biogerontology, cancer research, and regenerative medicine would highly benefit from the spectacular findings.

Keywords
Telomere shortening; telomerase; cellular ageing; chromatin remodeling; stem cells; differentiation; cell cycle

1. Telomeres and Telomerase Complex

We have never reached closer to understanding the phenomenon of ageing than the discovery of telomeres. The telomere was discovered by Hermann Muller in 1938, and described as a protecting structure at the ends of chromosomes by Barbara McClintock in 1940 [1]. Nearly half a century later, Blackburn and Gall published their study on a repeated tandem sequence in the chromosomal termini in *Tetrahymena thermophile*, characterized by 5'-CCCCAA-3' repeats [2]. The tandem hexanucleotide repeat, rich in guanosine with the sequence 5'GGGTTA-3', is conserved among most vertebrates [3].

The chromosomal termini, or simply telomeres (gr. *ending parts*), are composed of the tandem repeats with 3' guanine rich tails hanging over at both ends of the chromosome varying in lengths: in humans the G-overhangs are found between 100 and 280 nt (reviewed in [4]). The G-tails fold back to form a displacement loop in homologous recombination mechanism generating a Holliday junction, namely the t-loop [5, 6]. Telomeres also form a secondary DNA structure known as G-quadruplex (G_4) formed by guanine-guanine interactions of the two anti-parallel strands [7]. This highly variable DNA-DNA chromosome end structure is associated with a special protein complex, shelterin. Shelterin is a complex of six proteins contributing to its core activity, and binding either directly to the telomeric DNA (TRF1, TRF2, POT1) sequences or interact through protein-protein domains (TIN2, TPP1) [5, 8]. The telomere and shelterin complex protect the end of chromosomes from the double-strand break repair systems, especially from non-homologous end joining [9-13].

Therefore, the telomeres protect chromosomes from extremely disastrous recombination events. Non-homologous recombination end-joining (NHEJ) leads to the formation of dicentric or circular chromosomes resulting in a severe telomere dysfunction [14]. However, the main role of the telomere is to maintain the chromosome integrity during the replication process [15]. In some cells, this integrity is maintained through one more mechanism.

In the so-called 3rd phase phenomenon of the cell division described by Leonard Hayflick, a differentiated cell has certain mitotic capacity limit of 50 to 70 divisions, after which it becomes quiescent and incapable of further division [16]. This is due to the end replication problem: during the DNA replication, an approximately 200 nucleotides-long RNA primer on the lagging strand is unable to be replaced by a DNA polymerase, the Okazaki fragment [17-20]. The incomplete replication of the lagging strand is followed by about 50 – 200 bp telomeric DNA loss each cell division, which subsequently leads to critically shortened telomeres and the cell's growth arrest [21-23]. It became clear that strong correlation between the telomeres length, replication machinery and cellular ageing existed [24-26]. It had been hypothesized that an intrinsic regulatory pathway must act in favor for the cells with long telomeres. C. W. Greider and E. H. Blackburn identified a "novel" terminal transferase-like enzyme in *Tetrahymena* that had been able to synthesize and replicate the tandem 5'-TTGGGG-3' sequence on the shortened telomeres [27]. The ribonucleoprotein (RNP) enzymatic complex had been termed telomerase [28-30].

Telomerase is a multicomponent enzymatic complex capable of extending chromosomal termini by synthesizing *de novo* tandem repeats at two 3' ends of the same chromosome. In humans, telomerase is composed of two main components: human telomerase reverse transcriptase (hTERT) and human telomerase RNA component (hTERC or simply hTR) [15]. The RNA component (hTR) binds the 5' C-rich strand, and its 11-nucleotide sequence serves as the replication template for the reverse transcriptase [31-33].

Even though both components are crucial for telomerase activity, hTERT component alone is capable to extend the telomeres, as evidenced in multiple studies [34-37]. This phenomenon can be explained by high abundance of hTR molecules in most of the tissues, with long and short telomeres, while the hTERT component is downregulated in all cells but embryonic, adult germ cells, lymphocytes, and peripheral blood T and B cells [38, 39-43]. The convoluted cellular control mechanisms of the hTERT expression secure the cell from stepping into carcinogenesis pathway; however, its high mRNA levels in the developing embryo cells indicate that telomerase is an inherent and crucial factor [44, 45].

2. Theory of Biological Ageing: Somatic Cells vs Stem Cells

The origin of life and its ending have more in common than most could think of, and at some point they meet halfway. If cellular ageing could be defined by only two parameters: 1) loss of mitotic potential and 2) shortened telomeres – then the embryonic stem cell (ES) stands the opposite and might be considered "immortal." Indeed, *stricte* physical symptoms of ageing body are reflected by incapability of stem cells located in the organs' niches to replace dying somatic cells [46]. Moreover, it had been shown that the self-renewal capacity of the niches' stem cells is limited and also affected by truncated telomeres, and global cell cycle dysregulation [47].

Stem cells are defined as cells with the capacity of self-renewal and the ability to differentiate into multiple cell lineages [48]. Embryonic stem cells have been considered as pluripotent cells, with the ability to generate each cell line except for the extraembryonic tissues. This point of view changed when a series of studies had proven it wrong [49]: The embryonic stem cells, when treated with MEK (MAPK/ERK) and Gsk3 (glycogen synthase kinase 3) inhibitory procedure, they maintain their pluripotency, while some fractions sustain their totipotency. However, once a stem cell enters the path of differentiation and subsequent determination, it becomes a "mortal" somatic cell. It has been shown that the telomerase might function as one of the most important molecular switches in this mechanism [44].

The rearrangement of the "stemness" identity into the somatic is the driving force in all cells of a developing embryo. Because of the complexity of the entire dynamic system of the embryo, tracking all virtual changes within its cells is challenging. The most convenient approach in studying the morphogenetic changes of the developing embryo in its entirety is the view from the genome (VFG). This is the view of the genome network with respect to all nodes, circuits, subcircuits, and components (proteomic and genomic) at particular time [50]. The networks for specific body parts are composed of specific subcircuit components, namely transcription factors and their effectors (genes regulated at precise nodes and domains) [45, 51]. With the progress of embryo development, the distinct clusters of transcription factors constitute and determine distinct body parts networks (lineages) until they reach the *maximum saturation* point of specification and lineage determination, when there is only one lineage possible to choose for the

cell (unidirectional and progressive differentiation) [51]. Therefore, the stem cells found in distinct tissues are capable to differentiate only into this tissue – they become the unipotent stem cells, though with high plasticity. Although a common term, stem cells researchers are divided whether a *unipotent* stem cell exists at all; the data indicates that most of these cells are able to differentiate into more than just one lineage and the term *unipotent* does not necessarily imply their actual role [52-54].

From this, following conclusions can be drawn: 1) Differentiation is a multicomponent and multilevel process forwarded by subsequent and reciprocal interactions between transcription factors and target genes. 2) As it progresses, the stem cells change their biochemical states (on the basis of the genomic networks) by losing their plasticity and being coursed into more and more specified lineages. 3) The infinite number of doublings is changed to *finite* as they become fully the determined somatic cells. 4) One of the main cellular markers of their mortality are shortened telomeres, silenced expression of telomerase, and the resulting Hayflick limit.

This is a very simplified schema of the extremely convoluted process of the embryo development, genomic and environmental interactions, and the changes occurring in the developing and somatic cells, but if properly focused and addressed, leads to an interesting observation. Could the telomerase downregulation, consequential telomeres shortening, and the differentiated state of the cell be coupled? If so, what kind of mechanisms could be involved and what is the exact relation between these processes?

3. Regulation of Human Telomerase Expression

The *hTERT* locus is positioned on chromosome 5 (5p15.33), extending over 40 kb with 16 exons and 15 introns, 2 Mb away from the telomeric region [55]. It has been observed that the telomerase negative cells still generate differently spliced variants of the polymerase, with α-spliced (lacking 36 nucleotides from the 5' end of exon 6) and β-spliced (lacking exons 7 and 8) variants being the most widespread [15]. Recently it has been shown that cellular ageing and oncogenic processes are coupled with unique chromatin looping directed by telomeres; chromatin of the promoter of hTERT becomes more permissive for transcription elements (through epigenetic modifications) producing truncated and mostly inactive spliced variants of hTERT, thus protecting the cells from perilous telomerase activity [56]. Due to the limited scope of this article, more information on the alternatively spliced variants of the hTERT gene can be found in [41, 57].

Interestingly, the *hTERT* promoter lacks both TATA and CAAT boxes, but is marked by large CpG islands, including two canonical CACGTG (E-box) sequences [15, 58]. The CpG islands make the *hTERT* promoter susceptible to DNA methylation at the C residues [59]. Besides, the promoter has multiple binding sites for a great number of *trans*-regulatory elements regulating its expression state. Overall, the telomerase activity is controlled almost entirely by the transcription regulation of hTERT via epigenetic and *trans*-regulatory mechanisms. They briefly will be described and summarized below.

Methylation is the most prevalent epigenetic modification at the *hTERT* promoter, although not unambiguous [60, 61]. In retinoic acid-induced HL60 differentiated and human teratoma cells, *hTERT* promoter methylation pattern has its corresponding effect on the decreased hTERT expression [62], while in other studies on cancerous cells the methylation patterns have not always correlated with their telomerase expression levels [63-66].

Similarly, histones acetylation usually is related to an increased gene activity [67]. Even though downregulated hTERT in most somatic cells is associated with hypoacetylated core histones, this is not always true [68]. It is also known that DNA methylation and histone acetylation are coupled in the telomerase regulation machinery. Methyl-CpG biding proteins, such as MBD1 and MeCP2, act as mediators between those two processes as evidenced in a synergistic co-play between DNMT (DNA methyltransferase) and HDAC (histone deacetylase) repressor complexes recruited by PCNA (proliferating cell nuclear antigen) and interacting with p21 cell cycle regulator [69].

The transcription factor control over telomerase expression is even more intricate with the following observations. The core example of the whole network of regulators is heterodimeric E-box-binding c-Myc/Max complex, which is highly abundant at the *hTERT* promoter, and regulates its transcriptional activity [15, 70-72]. Human papillomavirus 16's E6 protein is another significant activating factor shown to activate telomerase in human keratinocytes and mammary epithelial cells [73]. As the endometrium cells are telomerase positive, due to the proliferating epithelium cells, it is not surprising that steroidal hormones, including estrogen, positively affect the hTERT expression as well [74, 75]. It has been demonstrated that the most engaged initiation site in the *hTERT* promoter is the GC-box for zinc finger far-flung Sp1 transcription factor, which cooperates with c-Myc in triggering telomerase activity [76].

On the contrary, the vastness of repression mechanisms and elements is exceeding over the telomerase activating regulators. Another heterodimer, Mad1/Max represses the *hTERT* transcription through histone deacetylation, and the switch between the two complexes, c-Myc/Max and Mad1/Max, depends the complexes' members' instantaneous concentration [77]. Most cell cycle negative regulators are involved in silencing the *hTERT* promoter, and this includes p16, p21, E2F, and p53 (mostly through Sp1 downregulation) [78-81]. And finally, the differentiation factors are also actively engaged in the hTERT downregulation, just to count TGF-β1, Activin, and BMP7, with well-studied telomerase regulating properties [82-85]. The Nature equipped cells with an impressive tool box for silencing the telomerase activity making sure that the enzyme would become quiescent by all means necessary.

More than this, there are other nodes of control over the telomerase activity, including posttranslational modification of the enzyme and the shelterin complex with its six core components interacting with other proteins [8, 59, 80]. The control occurs at each level of the telomerase activity, affected by hundreds of various biological factors. Very often it is impossible to find a correlation or a pattern among all the overflowing data, and when such a correlation is finally grasped, it is crucial to point it out with proper justification found in the enormous literature collection, and then to prove it with proper experimental evidence. This article makes an exacting attempt to define an exact correlation between regulatory mechanisms of telomerase and differentiating processes in the developmental course. How could they be possibly explained before any experimental procedure is applied?

4. Biomolecular Relationship between Cellular Differentiation and Ageing

Knowing that pluripotent embryonic cells are telomerase positive, we could freely draw conclusion that all of them should have similar telomeres length. However, it has been shown that the telomere length in the oocytes and blastocysts differs greatly from the telomeres in the zygote, more than it had been expected [86]. While an average oocyte's telomere length has been

determined as ~ 11.12 kb and a blastocyst's as 12.22 kb, the average telomere length for the cleavage stage embryo is ~ 8.43 kb [87]. It is speculated that the alternative lengthening of telomeres (ALT) mechanism through chromosomal recombination in early embryo cells could yield in this discrepancy [88]. The ALT pathway has not yet been observed in human embryo cells, but it already has been shown that both mechanisms (telomerase-dependent and –independent) coexist in the same cell, which possibly could explain the differential telomeres length phenomenon in the human embryo cells [89, 90]. Moreover, it has been reported that self-renewal capacity of human embryonic stem cells (and iPSCs) is secured by the telomere length maintenance through chromosomal trimming, a similar mechanism observed in uni- and multicellular organisms [4, 79, 90].

Strong correlation between the differentiation status of the cell and telomerase activity is indisputable. Unfortunately, this correlation is frequently overlooked by researchers. Most notably, the majority of the data in this spectrum have been reported in the studies on cancerous cells (see below). Only recently, by reason of the induced pluripotent stem cells research outbreak, it has been evidenced that the telomerase activity increases in somatic cells induced to become pluripotent [91, 92]. Since the first successful iPSCs establishment, the four main pluripotency transcription factors (Oct3/4, klf4, Sox2, c-Myc) have been recognized as the main "stemness" gate keepers [93]. Now, it is known that c-Myc is a dispensable factor in activating telomerase expression in the induced cells [92]. It is still uncertain how exactly Oct4, klf4, Sox2, or Nanog affect the *hTERT* promoter. Most likely, local chromatin and histones remodeling is the primary explanation based on the chromatin studies, and additionally complex cell cycle and apoptotic pathways, incorporating a broader range of proteins that had never been expected to be involved in the regulation of telomerase activity [91, 92, 94-96]. Recently, researchers have found a klf4 binding site in the *hTERT* promoter stimulating its activity through the Wnt signaling [70, 97].

Multiple studies, especially on promyelocytic leukemic HL-60 cells, indicate that terminal differentiation induced by differentiating factors results in the telomerase-negative phenotype [98, 99-101]. Other components of the telomerase complex were not up- or downregulated, proving once again that hTERT is the rate-limiting agent [99]. Apart from well-established epigenetic modifications, factors such as a cell cycle stage (G_0 putatively limits the telomerase activity), "stemness" status of a cell (non-dividing stem cells do not express hTERT as well presented in [102]), and poorly studied *trans*-acting repressors (and activators), including WT1 transcription factor, might affect the transcription of the *hTERT* promoter and be responsible for telomerase activity [69, 94, 100]. Even more importantly, the *trans*-acting proteins may interact with the *hTERT* promoter prior to the chromatin changes [80], and their effect is performed mostly through silencing, although the studies are very often contradicting [103, 104].

One could speculate whether the downregulation in telomerase expression might be a trigger for terminal differentiation, but the available data indicates the opposite. Hence, it could be stated that the terminal differentiation of the cell is possible if and only if the telomerase activity is downregulated through the yet unknown regulatory mechanisms. Silencing the *hTERT* promoter might be one of the final cellular statements towards the terminal differentiation. A few reasons explain this assumption. First, telomerase is active in ~ 90% of all tumors meaning that its role in cellular homeostasis is rather compelling [38, 98, 105-107, 108]. Second, *hTERT* has binding sites for some of the cell cycle regulators as well as its chromatin remodelers are activated and silenced by other cell cycle *trans*-acting particles, showing that the telomerase activity regulation is

strongly coupled with the intracellular control mechanisms [69, 70, 94, 96]. Third, it has been shown that the *hTERT* locus might affect the activity of other genes, including genes of the cell cycle, through special telomere positional effect, and its chromatin regulation could be another prerequisite for intracellular stabilization [56, 109].

Shortening of telomeres leads to the cell's replicative senescence and in the ultimate cases, their fatal impairment [20, 110-112]. This process contributes to organ impairment and systemic aging [113-117]. Telomere shortening is not the defining cause of cellular ageing and biological death, but one of the most significant ones. As the data points out and as our understanding of the ageing processes gradually deepens, the telomerase complex is not an isolated genome regulatory module, but a far-reaching part of the entire intracellular regulatory system. It must be yet carefully studied to what definite extent the differentiation mechanisms are correlated with the telomerase activity control. Afterwards, the next logical step is to determine the clinical applications from the gained knowledge in a plethora of medical disciplines. However, all the present data indicates a strong correlation between the differentiation processes and the telomerase gene silencing, which could be concluded in rather a philosophical statement: Death starts there where life begins.

5. Conclusions

Terminal determination of the cells is necessary to preserve certain global epigenetic marks and direct the cells towards their biological fate. The genomic changes during this complex mechanism are indiscernibly known, and even less is known on how they affect the telomerase activity. Within the chain of consecutive processes taking place in a virtual embryonic cell, the genomic loops of *cis*- and *trans*-acting regulators transform the pluripotent stem cells into the well-defined, fully differentiated somatic cell. During these changes, the *hTERT* promoter has been observed to be modified as well indicating the intriguing role of the telomerase in the developing and differentiating cells. Properly defined factors coupling the regulatory pathways of cellular differentiation and telomerase activity could become of a great importance.

This knowledge could be used in a plethora of medical applications. First, identifying the differentiation factors directly involved in the telomerase regulation gives a chance to develop new clinical regulators of the telomerase promoter. Second, the newly recognized telomerase regulatory pathways could bring closer the understanding of some yet-unknown cancer development mechanisms. Third, the stem cells research and regenerative medicine fields might benefit in understanding the regulatory processes behind iPSCs and their improvement. The ongoing research on the telomerase and its components over the last thirty years has shown an incredible scope of the enzyme activities involved in the cellular homeostasis, contributing to more than just cellular ageing.

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Competing Interests

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References

1. McClintock B, Hill HE. The cytological identification of the chromosome associated with the RG linkage group in Zea mays. Genetics. 1931; 16: 175-190.
2. Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J Mol Biol. 1978; 120: 33-53.
3. Meyne J, Ratliff RL, MoYzIs RK. Conservation of the human telomere sequence (TTAGGG) n among vertebrates. P Natl Acad Sci. 1989; 86: 7049-7053.
4. Webb C J., Wu Y, Zakian VA. DNA repair at telomeres: keeping the ends intact. CSH Perspect Biol. 2013; 5: a012666.
5. de Lange T. T-loops and the origin of telomeres. Nat Rev Mol Cell Biol. 2004; 5: 323-329.
6. Griffith JD, Comeau L, Rosenfield S, Stansel RM, Bianchi A, Moss H, et al. Mammalian telomeres end in a large duplex loop. Cell. 1999; 97: 503-514.
7. Phillips K, Dauter Z, Murchie AIH, Lilley DMJ, Luisi B. The crystal structure of a parallel-stranded guanine tetraplex at 0.95 Å resolution1. J Mol Biol. 1997; 273: 171-182.
8. Palm W, de Lange T. How shelterin protects mammalian telomeres. Ann Rev Genet. 2008; 42: 301-334.
9. Denchi EL, de Lange T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. Nature. 2007; 448: 1068.
10. Hirano Y, Sugimoto K. Cdc13 telomere capping decreases Mec1 association but does not affect Tel1 association with DNA ends. Mol Biol Cell. 2007; 18: 2026-2036.
11. Flynn RL, Centore RC, Centore RJ, Rai R, Tse A, Zhou S, et al. TERRA and hnRNPA1 orchestrate an RPA-to-POT1 switch on telomeric single-stranded DNA. Nature. 2011; 471: 532-536.
12. Stansel RM, De Lange T, Griffith JD. T-loop assembly in vitro involves binding of TRF2 near the 3' telomeric overhang. EMBO J. 2001; 20: 5532-5540.
13. Ye J, Lenain C, Bauwens S, Rizzo A, Saint-Léger A, Poulet A, et al. TRF2 and apollo cooperate with topoisomerase 2α to protect human telomeres from replicative damage. Cell. 2010; 142: 230-242.
14. De Lange T. Shelterin: The protein complex that shapes and safeguards human telomeres. Gene Dev. 2005; 19: 2100-2110.
15. Cong YS, Wright WE, Shay JW. Human telomerase and its regulation. Microbiol Mol Biol Rev. 2002; 66: 407-425.

16. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res. 1961; 25: 585-621.
17. Szostak JW. Evolutionary conservation of the structure of eucaryotic telomeres. Recent Adv Yeast Mol Biol. 1982.
18. Olovnikov 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; 41: 181-190.
19. Watson JD. Origin of concatemeric T7DNA. Nat New Biol. 1972; 239: 197-201.
20. Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. Nature. 1990; 345: 458.
21. Lindsey J, McGill NI, Lindsey LA, Green DK, Cooke HJ. In vivo loss of telomeric repeats with age in humans. Mutat Res/DNAging. 1991; 256: 45-48.
22. Hastie ND, Dempster M, Dunlop MG, Thompson AM, Green DK, Allshire RC. Telomere reduction in human colorectal carcinoma and with ageing. Nature. 1990; 346: 866.
23. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell. 1985; 43: 405-413.
24. Greider CW, Blackburn EH. A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature. 1989; 337: 331.
25. Greider CW, Blackburn EH. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell. 1987; 51: 887-898.
26. Larson DD, Spangler EA, Blackburn EH. Dynamics of telomere length variation in Tetrahymena thermophila. Cell. 1987; 50: 477-483.
27. Turchi JJ, Huang L, Murante RS, Kim Y, Bambara RA. Enzymatic completion of mammalian lagging-strand DNA replication. P Natl Acad Sci. 1994; 91: 9803-9807.
28. Balakrishnan L, Bambara RA. Okazaki fragment metabolism. CSH Perspect Biol. 2013; 5: a010173.
29. Hubscher U, Seo YS. Replication of the lagging strand: a concert of at least 23 polypeptides. Mol Cells. 2001; 12: 149-157.
30. Turchi JJ, Bambara RA. Completion of mammalian lagging strand DNA replication using purified proteins. J Biol Chem. 1993; 268: 15136-15141.
31. Harrington L, Zhou W, McPhail T, Oulton R, Yeung DSK, Mar V, et al. Human telomerase contains evolutionarily conserved catalytic and structural subunits. Gene Dev. 1997; 11: 3109-3115.
32. Kilian A, Bowtell DDL, Abud HE, Hime GR, Venter DJ, Keese PK, et al. Isolation of a candidate human telomerase catalytic subunit gene, which reveals complex splicing patterns in different cell types. Hum Mol Genet. 1997; 6: 2011-2019.
33. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR. Reverse transcriptase motifs in the catalytic subunit of telomerase. Science. 1997; 276: 561-567.
34. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998; 279: 349-352.
35. Vaziri H, Benchimol S. Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. Curr Biol. 1998; 8: 279-282.
36. Yang J, Chang E, Cherry AM, Bangs CD, Oei Y, Bodnar A, et al. Human endothelial cell life extension by telomerase expression. J Biol Chem. 1999; 274: 26141-26148.

37. Cairney CJ, Keith WN. Telomerase redefined: Integrated regulation of hTR and hTERT for telomere maintenance and telomerase activity. Biochimie. 2008; 90: 13-23.
38. Yi X, Tesmer VM, Savre-Train I, Shay JW, Wright WE. Both transcriptional and posttranscriptional mechanisms regulate human telomerase template RNA levels. Mol Cell Biol. 1999; 19: 3989-3997.
39. Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, Chiu CP, et al. The RNA component of human telomerase. Science. 1995; 269: 1236-1241.
40. Avilion AA, Piatyszek MA, Gupta J, Shay JW, Bacchetti S, Greider CW. Human telomerase RNA and telomerase activity in immortal cell lines and tumor tissues. Cancer Res. 1996; 56: 645-650.
41. Ulaner GA, Hu JF, Vu TH, Giudice LC, Hoffman AR. Telomerase activity in human development is regulated by human telomerase reverse transcriptase (hTERT) transcription and by alternate splicing of hTERT transcripts. Cancer Res. 1998; 58: 4168-4172.
42. Liu K, Schoonmaker MM, Levine BL, June CH, Hodes RJ, Weng N. Constitutive and regulated expression of telomerase reverse transcriptase (hTERT) in human lymphocytes. P Natl Acad Sci. 1999; 96: 5147-5152.
43. Ramakrishnan S, Eppenberger U, Mueller H, Shinkai Y, Narayanan R. Expression profile of the putative catalytic subunit of the telomerase gene. Cancer Res. 1998; 58: 622-625.
44. Morrison SJ, Prowse KR, Ho P, Weissman IL. Telomerase activity in hematopoietic cells is associated with self-renewal potential. Immunity. 1996; 5: 207-216.
45. Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, et al. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell. 2005; 122: 947-956.
46. Liebich S. The biology of aging-selected aspects. Postepy Biol Komorki. 2016; 43: 563-578.
47. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nat Rev Mol Cell Biol. 2007; 8: 703.
48. Smith AG. Embryo-derived stem cells: of mice and men. Ann Rev Cell Dev Biol. 2001; 17: 435-462.
49. Morgani SM, Canham MA, Nichols J, Sharov AA, Migueles RP, Ko MSH, et al. Totipotent embryonic stem cells arise in ground-state culture conditions. Cell Rep. 2013; 3: 1945-1957.
50. Howard ML, Davidson EH. cis-Regulatory control circuits in development. Dev Biol. 2004; 271: 109-118.
51. Davidson EH. Spatial mechanisms of gene regulation in metazoan embryos. Development. 1991; 113: 1-26.
52. Eisenberg LM, Eisenberg CA. Stem cell plasticity, cell fusion, and transdifferentiation. Birth Defects Res C. 2003; 69: 209-218.
53. Slack JMW. Stem cells in epithelial tissues. Science. 2000; 287: 1431-1433.
54. Jaenisch R, Young R. Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. Cell. 2008; 132: 567-582.
55. Cong YS, Wen J, Bacchetti S. The human telomerase catalytic subunit hTERT: Organization of the gene and characterization of the promoter. Hum Mol Genet. 1999; 8: 137-142.
56. Kim W, Ludlow AT, Min J, Robin JD, Stadler G, Mender I, et al. Regulation of the human telomerase gene TERT by telomere position effect—over long distances (TPE-OLD): Implications for aging and cancer. PLoS Biol. 2016; 14: e2000016.

57. Yi X, White DM, Aisner DL, Baur JA, Wright WE, Shay JW. An alternate splicing variant of the human telomerase catalytic subunit inhibits telomerase activity. Neoplasia. 2000; 2: 433-440.
58. Iliopoulos D, Satra M, Drakaki A, Poultsides GA, Tsezou A. Epigenetic regulation of hTERT promoter in hepatocellular carcinomas. Int J Oncol. 2009; 34: 391-399.
59. Lai SR, Phipps SMO, Liu L, Andrews LG, Tollefsbol TO. Epigenetic control of telomerase and modes of telomere maintenance in aging and abnormal systems. Front Biosci. 2005; 10: 96.
60. Widschwendter A, Müller HM, Fiegl H, Ivarsson L, Wiedemair A, Müller-Holzner E, et al. DNA methylation in serum and tumors of cervical cancer patients. Clin Cancer Res. 2004; 10: 565-571.
61. Guilleret I, Benhattar J. Demethylation of the human telomerase catalytic subunit (hTERT) gene promoter reduced hTERT expression and telomerase activity and shortened telomeres. Exp Cell Res. 2003; 289: 326-334.
62. Guilleret I, Yan P, Grange F, Braunschweig R, Bosman FT, Benhattar J. Hypermethylation of the human telomerase catalytic subunit (hTERT) gene correlates with telomerase activity. Int J Cancer. 2002; 101: 335-341.
63. Lopatina NG, Poole JC, Saldanha SN, Hansen NJ, Key JS, Pita MA, et al. Control mechanisms in the regulation of telomerase reverse transcriptase expression in differentiating human teratocarcinoma cells. Biochem Bioph Res Commun. 2003; 306: 650-659.
64. Kitagawa Y, Kyo S, Takakura M, Kanaya T, Koshida K, Namiki M, et al. Demethylating reagent 5-azacytidine inhibits telomerase activity in human prostate cancer cells through transcriptional repression of hTERT. Clin Cancer Res. 2000; 6: 2868-2875.
65. Devereux TR, Horikawa I, Anna CH, Annab LA, Afshari CA, Barrett JC. DNA methylation analysis of the promoter region of the human telomerase reverse transcriptase (hTERT) gene. Cancer Res. 1999; 59: 6087-6090.
66. Guilleret I, Benhattar J. Demethylation of the human telomerase catalytic subunit (hTERT) gene promoter reduced hTERT expression and telomerase activity and shortened telomeres. Exp Cell Res. 2003; 289: 326-334.
67. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997; 389: 349.
68. Takakura M, Kyo S, Sowa Y, Wang Z, Yatabe N, Maida Y, et al. Telomerase activation by histone deacetylase inhibitor in normal cells. Nucleic Acids Res. 2001; 29: 3006-3011.
69. Liu L, Saldanha SN, Pate MS, Andrews LG, Tollefsbol TO. Epigenetic regulation of human telomerase reverse transcriptase promoter activity during cellular differentiation. Gene Chromosome Cancer. 2004; 41: 26-37.
70. Hoffmeyer K, Raggioli A, Rudloff S, Anton R, Hierholzer A, Del Valle I, et al. Wnt/β-catenin signaling regulates telomerase in stem cells and cancer cells. Science. 2012; 336: 1549-1554.
71. Poole JC, Andrews LG, Tollefsbol TO. Activity, function, and gene regulation of the catalytic subunit of telomerase (hTERT). Gene. 2001; 269: 1-12.
72. Wang J, Xie LY, Allan S, Beach D, Hannon GJ. Myc activates telomerase. Gene Dev. 1998; 12: 1769-1774.
73. Klingelhutz AJ, Foster SA, McDougall JK. Telomerase activation by the E6 gene product of human papillomavirus type 16. Nature. 1996; 380: 79.
74. Kyo S, Takakura M, Kanaya T, Zhuo W, Fujimoto K, Nishio Y, et al. Estrogen activates telomerase. Cancer Res. 1999; 59: 5917-5921.

75. Jaskelioff M, Muller FL, Paik JH, Thomas E, Jiang S, Adams AC, et al. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011; 469: 102.
76. Kyo S, Takakura M, Taira T, Kanaya T, Itoh H, Yutsudo M, et al. Sp1 cooperates with c-Myc to activate transcription of the human telomerase reverse transcriptase gene (hTERT). Nucleic Acids Res. 2000; 28: 669-677.
77. Xu D, Popov N, Hou M, Wang Q, Björkholm M, Gruber A, et al. Switch from Myc/Max to Mad1/Max binding and decrease in histone acetylation at the telomerase reverse transcriptase promoter during differentiation of HL60 cells. P Natl Acad Sci. 2001; 98: 3826-3831.
78. Zhu XD, Küster B, Mann M, Petrini JHJ, de Lange T. Cell-cycle-regulated association of RAD50/MRE11/NBS1 with TRF2 and human telomeres. Nat Genet. 2000; 25: 347.
79. Greider CW. Telomere length regulation. Ann Rev Biochem. 1996; 65: 337-365.
80. Lin SY, Elledge SJ. Multiple tumor suppressor pathways negatively regulate telomerase. Cell. 2003; 113: 881-889.
81. Shats I, Milyavsky M, Tang X, Stambolsky P, Erez N, Brosh R, et al. p53-dependent down-regulation of telomerase is mediated by p21waf1. J Biol Chem. 2004; 279: 50976-50985.
82. Xiao L, Yuan X, Sharkis SJ. Activin A maintains self-renewal and regulates fibroblast growth factor, Wnt, and bone morphogenic protein pathways in human embryonic stem cells. Stem Cells. 2006; 24: 1476-1486.
83. Rama, SYS, Rao AJ. Regulation of telomerase during human placental differentiation: A role for TGFβ1. Mol Cell Endocrinol. 2001; 182: 233-248.
84. Maellaro E, Pacenti L, Del Bello B, Valentini MA, Mangiavacchi P, De Felice C, et al. Different effects of interferon-α on melanoma cell lines: A study on telomerase reverse transcriptase, telomerase activity and apoptosis. Brit J Dermatol. 2003; 148: 1115-1124.
85. Li H, Xu D, Li J, Berndt MC, Liu JP. Transforming growth factor β suppresses human telomerase reverse transcriptase (hTERT) by Smad3 interactions with c-Myc and the hTERT gene. J Biol Chem. 2006; 281: 25588-25600.
86. Wright DL, Jones EL, Mayer JF, Oehninger S, Gibbons WE, Lanzendorf SE. Characterization of telomerase activity in the human oocyte and preimplantation embryo. Mol Hum Reprod. 2001; 7: 947-955.
87. Turner S, Wong HP, Rai J, Hartshorne GM. Telomere lengths in human oocytes, cleavage stage embryos and blastocysts. Mol Hum Reprod. 2010; 16: 685-694.
88. Bryan TM, Englezou A, Dalla-Pozza L, Dunham MA, Reddel RR. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. Nat Med. 1997; 3: 1271.
89. Guiducci C, Cerone MA, Bacchetti S. Expression of mutant telomerase in immortal telomerase-negative human cells results in cell cycle deregulation, nuclear and chromosomal abnormalities and rapid loss of viability. Oncogene. 2001; 20: 714.
90. Rivera T, Haggblom C, Cosconati S, Karlseder J. A balance between elongation and trimming regulates telomere stability in stem cells. Nat Struct Mol Biol. 2017; 24: 30.
91. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. 2007; 318: 1917-1920.

92. Marion RM, Strati K, Li H, Tejera A, Schoeftner S, Ortega S, et al. Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. Cell Stem Cell. 2009; 4: 141-154.
93. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007; 131: 861-872.
94. Zhu J, Zhao Y, Wang S. Chromatin and epigenetic regulation of the telomerase reverse transcriptase gene. Protein Cell. 2010; 1: 22-32.
95. Chen T, Du J, Lu G. Cell growth arrest and apoptosis induced by Oct4 or Nanog knockdown in mouse embryonic stem cells: A possible role of Trp53. Mol Biol Rep. 2012; 39: 1855-1861.
96. Wetterau LA, Francis MJ, Ma L, Cohen P. Insulin-like growth factor I stimulates telomerase activity in prostate cancer cells. J Clin Endocrinol Metab. 2003; 88: 3354-3359.
97. Wong CW, Hou PS, Tseng SF, Chien CL, Wu KJ, Chen HF, et al. Krüppel-like transcription factor 4 contributes to maintenance of telomerase activity in stem cells. Stem Cells. 2010; 28: 1510-1517.
98. Meyerson M, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, et al. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell. 1997; 90: 785-795.
99. Xu D, Gruber A, Björkholm M, Peterson C, Pisa P. Suppression of telomerase reverse transcriptase (hTERT) expression in differentiated HL-60 cells: Regulatory mechanisms. Brit J Cancer. 1999; 80: 1156.
100. Holt SE, Wright WE, Shay JW. Regulation of telomerase activity in immortal cell lines. Mol Cell Biol. 1996; 16: 2932-2939.
101. Zhang W, Piatyszek MA, Kobayashi T, Estey E, Andreeff M, Deisseroth AB, et al. Telomerase activity in human acute myelogenous leukemia: Inhibition of telomerase activity by differentiation-inducing agents. Clin Cancer Res. 1996; 2: 799-803.
102. Ravindranath N, Dalal R, Solomon B, Djakiew D, Dym M. Loss of telomerase activity during male germ cell differentiation. Endocrinology. 1997; 138: 4026-4029.
103. Hou M, Wang X, Popov N, Zhang A, Zhao X, Zhou R, et al. The histone deacetylase inhibitor trichostatin A derepresses the telomerase reverse transcriptase (hTERT) gene in human cells. Exp Cell Res. 2002; 274: 25-34.
104. Wang S, Zhu J. Evidence for a relief of repression mechanism for activation of the human telomerase reverse transcriptase promoter. J Biol Chem. 2003; 278: 18842-18850.
105. Kim NW, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, et al. Specific association of human telomerase activity with immortal cells and cancer. Science. 1994; 266: 2011-2015.
106. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. Carcinogenesis. 2009; 31: 9-18.
107. Greider CW, Blackburn EH. Telomeres, telomerase and cancer. Sci Am. 1996; 274: 92-97.
108. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100: 57-70.
109. Wood AM, Rendtlew Danielsen JM, Lucas CA, Rice EL, Scalzo D, Shimi T, et al. TRF2 and lamin A/C interact to facilitate the functional organization of chromosome ends. Nat Commun. 2014; 5: 5467.
110. de Lange T. Activation of telomerase in a human tumor. P Natl Acad Sci. 1994; 91: 2882-2885.
111. Blackburn EH. Telomere states and cell fates. Nature. 2000; 408: 53.

112. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet. 2005; 6: 611.
113. Blasco MA, Lee HW, Hande MP, Samper E, Lansdorp PM, DePinho RA, et al. Telomere shortening and tumor formation by mouse cells lacking telomerase RNA. Cell. 1997; 91: 25-34.
114. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, et al. Longevity, stress response, and cancer in aging telomerase-deficient mice. Cell. 1999; 96: 701-712.
115. Artandi SE, DePinho RA. Mice without telomerase: what can they teach us about human cancer?. Nat Med. 2000; 6: 852.
116. Blackburn EH, Epel ES, Lin J. Human telomere biology: A contributory and interactive factor in aging, disease risks, and protection. Science. 2015; 350: 1193-1198.
117. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years or older. The Lancet. 2003; 361: 393-395.