Scientific Research Publishing

Journal of Biosciences and Medicines, 2022, 10, 221-230
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Research Progress of Parasite Telomerase and Its Interacting Proteins

Kaisong Hu, Jiaqi Zhang, Jingtong Zheng*

Department of Pathogen Biology, College of Basic Medical Sciences, Jilin University, Changchun, China
Email: *zhengjingtong@jlu.edu.cn, *wszjt1@163.com

How to cite this paper: Hu, K.S., Zhang, J.Q. and Zheng, J.T. (2022) Research Progress of Parasite Telomerase and Its Interacting Proteins. *Journal of Biosciences and Medicines*, **10**, 221-230.
https://doi.org/10.4236/jbm.2022.1010019

Received: September 26, 2022
Accepted: October 28, 2022
Published: October 31, 2022

Abstract

Telomerase is a nucleoprotein that contains reverse transcriptase activity. It is reversely transcribed into DNA at the end of its chromosome using its own RNA as a template to ensure the length and stability of telomeres. Its physiological function is closely related to the life process of cells. In recent years, the research on telomeres and telomerase has become a new hotspot, people have a more in-depth research and scientific understanding of their structures and functions, and scientists have also attached great importance to the research progress of telomerase increasingly. Researchers in parasite-related research have successively discovered a large number of telomere and telomerase sequences in a variety of different parasites. These achievements have greatly facilitated the scientific research of diseases caused by parasitic infections, providing a solid research basis for the preparation of new drugs, while enriching the treatment protocols of parasitic diseases, and opening new worlds for the prevention of certain diseases and epidemic transmission. This paper reviews the current research progress of telomerase.

Keywords

Telomerase, Telomeres, Parasites, Research Progress

1. Introduction

A telomere is a special nucleoprotein complex located at the end of eukaryotic chromosomes and plays an important role in chromosome positioning, replication, protection and control of cell growth and lifespan [1] [2]. It is composed of many short non-coding tandem repeat units, and the repeat sequences of telomeres vary widely among different organisms. The 3' of the telomeric DNA repeats of some eukaryotic parasites is a single-stranded overhang rich in G, and the single-stranded overhang replaces its own strand by folding back to the

double-stranded region of the telomere, and pairs with the complementary strand to form a distinct "T-loop" structure. Such "T-loop" structure will form a "D-loop-T-loop" [3] jointly with the "D-loop" sequence which is formed by the replacement. Studies have shown that when the Ser365 telomere protein complex of the telomere-binding protein TRF2 is dephosphorylated in S phase, a gap occurs, prompting the RTEL1 helicase to enter and unwind the "T-loop" to facilitate telomere replication. When RTEL1 needs to escape from the telomere, it needs to rephosphorylate Ser365 of TRF2 except in S phase. This phosphorylation protects the "T-loop" from promiscuous unwinding and inappropriate activation of ATM on the one hand, and eliminates replication conflicts within the telomere and throughout the genome's DNA secondary structure on the other hand, revealing the kinetic mechanism that the telomeric "T-loop" protects chromosomes, namely the telomere is a special structure composed of non-coding DNA that protect the ends of chromosomes. The special structure comes in two forms. When the single-stranded DNA is closed, the lasso-like "T-loop" structure buries the DNA end in the telomere and obscures it so that it cannot be detected as DNA damage. It is because the T loop is completed by the spatial folding of telomeres, so the T loop can be folded or unfolded by changing the spatial structure of the telomere. When the DNA sequence is opened, telomere maintenance and elongation reactions can be carried out [4].

There have been some research reports abroad on the research of parasite telomeres of different species. At present, the telomeric DNA repeats of Trichina, Trichomonas vaginalis, Giardia duodenalis, Plasmodium falciparum, Leishmania, Trypanosoma cruzi, Pneumocystis, roundworm and paraascaris have been identified [5]-[12]. At present, it has been found that the repeat sequences of most ciliates and mammalian cells are quite regular, but there are some other organisms whose repeat sequences are irregular. For example, the telomere sequence of yeast is C1-3A/TG1-3 [13]. Besides, there are also insertions of fragments such as TTTGGG in the repeat sequences in some paramecia [14]. In addition to repeat sequences, the length of telomeric DNA also differs significantly between species. Specifically, the telomere length of Euplotes is 28 bp [15], that of Giardia is 0.5 - 2.5 kb [16], and the same of Coccidia is 2 - 5 kb [17]. The difference causes and regulatory mechanisms of telomere sequences in these species need to be further investigated. Studies have shown that the normal aging process is associated with shortening of telomeres, while the shortening of telomeres is further associated with mortality and aging-related diseases. At present, there are three common telomere length determination methods:

1) Terminal Restriction Fragment Analysis (TRF) [18]: TRF is the earliest and most classical method for telomere length determination. This method is to assess the average telomere length of a population of cells. According to the specific and repetitive nature of the telomere sequence, the genomic DNA is digested by a set of restriction enzymes lacking telomere recognition sites. Since the telomeric DNA is not cut, its length can be determined by Southern blotting. TRF requires a large number of DNAs and takes a long time, so it is gradually

replaced by other methods [19].

2) Quantitative Fluorescence in Situ Hybridization (Q-FISH) [20]: Q-FISH is to determine the telomere length on chromosomes by hybridizing with fluorescently labeled nucleic acid probes to denatured telomeric DNA repeats in metaphase cells. This method enables the study of telomere length in cells with low cell mass, but the limitation is that it is difficult to detect senescent cells, and this method is not applicable for the telomeres of other cells such as non-dividing cells and highly aberrant cells [21].

3) Quantitative PCR (qPCR) [22]: In order to overcome the limitation that the TRF method requires a large number of DNAs, a polymerase chain reaction-based telomere length determination method has been developed, which significantly reduces the required DNA content and can amplify the DNA telomeres and single-copy genes in the same tube at the same time, so that telomere length is analyzed by parallel control, which is called MMqPCR method. The MMqPCR method allows the comparison of DNA samples sourced from different qualities, and the difference in the amplification efficiency of telomeres and single-copy genes themselves can be controlled by ratio. In 2011, O'Callghan and Fenech also improved the qPCR method, and the improved qPCR method is called aTLqpPCR, which uses a standard curve to assess the length of telomere base pairs in samples [23].

There are two main ways to maintain the length of telomere DNA, including the alternative lengthening of telomeres (ALT) [24], and the telomerase regulation mechanism [23]. The ALT mechanism is characterized by a "(CCCTAA)n" like C-loop structure, and the abundance of telomeric C-loop DNA is 1000-fold higher than in cells not regulated by the ALT mechanism. At present, the ALT pathway has not been found in parasites, therefore, the regulation mechanism of telomeres in parasites should be dominated by telomerase regulation.

2. Telomerase

The structure of telomerase is rather special. De novo synthesis of telomeric repeats at the ends of chromosomes by telomerase requires the reverse transcriptase activity of the telomerase reverse transcriptase (TERT) subunit, an internal RNA (e.g. human TER or hTR) template embedded in the telomerase RNA, and a telomere-associated protein (TAP) [25], and telomerase can add multiple telomeric repeats to a DNA substrate before dissociation. This property is known as repeat addition process [26]. These peculiar characteristics have led to numerous phylogenetic, genetic, biochemical and structural studies to determine how TERT and TER coordinate telomerase DNA synthesis and how this is achieved through a process of repetitive addition [27]. Among different eukaryotes, TERT has four conserved domains: the telomerase-essential N-terminal (TEN) domain, the telomerase RNA-binding domain (TRBD), the reverse transcriptase (RT) domain, and C-terminal extension (CTE) domain [28]. Specifically, the TEN domain has both DNA and RNA binding properties. DNA bind-

ing facilitates telomerase to be loaded onto chromosomes, whereas RNA binding is nonspecific [29]. The RT domain has a telomerase active site and is thought to be involved in loose binding to the RNA template. The TRBD domain is located between the TEN and RT domains. Unlike the TEN domain, it is highly conserved across phylogenetic groups and is required for telomerase function both in vitro and in vivo. TRBD contains key marker motifs (CP-motif and T-motif) that involve the recognition and binding of RNAs. The recent reports about high-resolution molecular structures of some TR and protein-RNA complexes have significantly improved the understanding of TR structures [30].

The research on the parasite TERT started relatively late. Malik *et al.* (2000) discovered the structure of telomerase in Giardia duodenalis, and the study showed that compared with TERT from other known organisms, Giardia duodenalis lacked telomerase T motif [31]. In 2009, Yang Guilian successfully cloned Eimeria tenella TERT 4688 bp full-length cDNA sequence [32]. In 2011, Cai Yanan successfully cloned the telomerase TERT gene of trichina with a full-length sequence of 3788 bp [33]. In 2013, Zhao Na *et al.* cloned the TERT gene of Trypanosoma evansi [34]. In addition, TERT sequences of various parasites such as Toxoplasma gondii, Trypanosoma cruzi, Trypanosoma brucei, Caenorhabditis elegans, and Schistosoma japonicum have been identified [35]. And the telomerase research on some parasites, such as Clonorchis sinensis and amoeba histolytica, is still in the research stage.

2.1. Function of Telomerases

Current studies believe that telomere shortening or other non-telomere pathways that initiate senescence play a key role in cellular senescence, which is helpful to manage DNA instability and prevent abnormal cells from appearing, as well as acts as a barrier to tumor development, while it is chromosomally unstable, and will result in genomic rearrangements that cause tumorigenesis due to telomere attrition, and dramatic cell division [36]. Gene mutation is a key factor indicating an enhanced phenotype of aging; mutations in TERT, TERC, or other telomere-preserving genes, can lead to a range of diseases, such as dyskeratosis congenita, cryptogenic cirrhosis, Revesz syndrome, etc. [33].

2.2. Functions of Telomerase-Interacting Proteins

Protein interaction is constituted by the interaction of individual proteins with each other to participate in all links of life processes such as biological signal transmission, gene expression regulation, energy and material metabolism, and cell cycle regulation. The interaction of proteins in biological systems is of great significance for understanding the working principle of proteins in biological systems, understanding the response mechanism of biological signals and energy metabolism under special physiological conditions such as diseases, and understanding the functional connections between proteins [36]. Many studies have shown that the post-translational regulation of telomerase activity may be

through reversible phosphorylation of specific Ser/Thr or Tyr residues on the catalytic subunit of TERT [37]. Phosphorylation sites exist in TERT from plants to animals, some of which are critical, and the phosphorylation of the sites will affect telomerase activity. For example, in response to radiation injury, c-Ab1 tyrosine kinase phosphorylates TERT-specific tyrosine residues, resulting in a 3-fold reduction in telomerase activity, and mice lacking c-Ab1 show increased telomerase activity and elongation of telomeres. Overexpression of c-Ab1 inhibited cell growth by inducing cell cycle arrest [36].

With the in-depth study of telomerase, a variety of telomerase-related proteins have been reported and they are found to play an important role in the regulation of telomerase activity [38]. In human cells, hTERT can mediate the protective function at chromosome ends of TERT in tumors by interacting with TPP1 [39]; hTERT can participate in the regulation of telomerase activity by interacting with DDRGK1 [40]. Tetrahymena p65 and p45, as part of telomerase, play an essential role in telomere maintenance [41]; in Trypanosoma brucei, YTH technology was used to screen out the RAP1 protein that interacts with TRF [38]; in Plasmodium falciparum, Soundara *et al.* used YTH technology to study the molecular chaperone protein interaction network [42]. Our research team identified the Giardia TERT-interacting protein ZFD by yeast two-hybrid screening, pull-down and co-immunoprecipitation techniques in the early stage of the study, and then explored the function of ZFD in the regulation of telomerase using the viral vector-mediated hammerhead ribozyme. The results show that the reduction of ZFD mRNA can cause the reduction of the reproductive rate of Giardia and the reduction of the number of worms. Besides, it was analyzed that the reduction of ZFD mRNA can reduce telomerase activity and make telomeres shorter through the determination of telomerase activity and telomere length [43]. Mingzhu Research successfully screened the interaction protein Hsp90 of TRBD in the telomerase RNA-binding domain of Giardia duodenalis using the yeast two-hybrid system, and verified the existence of interaction between TRBD and Hsp90 by bimolecular fluorescence technology, co-immunoprecipitation technology and GST-Pull down technology. Meanwhile, it also used the immunofluorescence technique to observe the distribution of TRBD and Hsp90 in Giardia, and then used the Duolink PLA technique to confirm that the two interacted in Giardia and co-localized on the Giardia binuclear [44].

2.3. Telomerase Activity Assay

The detection of telomerase activity originated from the TRAP method established by Kim in 46 [45]. The main principle of this method is as follows: First, a TS is synthesized as an upstream primer, and telomerase binds to the GTT at the end of TS and synthesizes into a sequence. Then, a new base repeat sequence will be synthesized through each translocation. After telomerase is inactivated, CX is added as a downstream primer. Through multiple denaturation annealing extension, the telomerase extension product is amplified. Finally, the activity of te-

lomerase was detected by gel electrophoresis. Positive results show ladder-like bands on gel electrophoresis, and the number and colors of the bands indicate the level of telomerase activity [45]. At present, the commonly-used telomerase activity detection method is to combine PCR with Elisa methods. In the first step, PCR amplification is performed. In the second step, the PCR product is divided into two equal parts, and then denatured hybridization is performed for the telomeric repeat sequence and the internal standards, respectively. The resulting product is immobilized on a streptavidin-coated microplate via a biotin tag. The results were then checked with the horseradish peroxidase-conjugated antibody digoxin [46]. Zhu Jinrui proposed a chemiluminescence instant detection method for telomerase activity using a handheld luminescence detector as a detection tool [47]. The main research contents are as follows: 1. The sensitive detection of telomerase activity by chemiluminescence real-time detection method established the instant detection method of telomerase activity chemiluminescence for the first time by using a handheld luminescence detector as a detection tool, based on horseradish peroxidase (HRP) catalysis of luminol/hydrogen peroxide/piodophenol (luminol/H_2O_2/PIP) chemiluminescence reaction. Under this method, Biotin-labeled telomeric DNA primers (TS) are modified to the surface of streptavidin-functionalized magnetic beads by streptavidin-biotin-specific interaction. Telomerase prolongs TS to form an extension product TEP containing multiple TTAGGG repeat units. Each long TEP chain can hybridize with multiple short horseradish peroxidase-labeled complementary DNA strands (HRP-cDNA), thereby enriching a large amount of HRP on the surface of the magnetic beads. After magnetic separation and washing, the specifically enriched HRP on the magnetic beads catalyzes the chemiluminescence reaction of luminol/H_2O_2/PIP.

The primers in the kit species are usually mammalian primers, and there are few special parasite telomere detection kits. Therefore, the upstream and downstream primers in the kit can be modified according to the principle of the TRAP method for parasite research. The results show that the band is clear, and they can be used for the detection of parasite telomerase activity [48].

3. Conclusion

Telomerase is an important and complex component of eukaryotic cell biology that has been studied for many years and is involved in processes ranging from cellular senescence, stress response, homeostasis, DNA repair and tumorigenesis. It has become a popular target for gene therapy, immunotherapy and chemotherapy in cancer and other diseases. In this paper, we provide an up-to-date overview of the origin, biogenesis, regulation, function, and experimental detection of parasite telomerase. To gain a deeper understanding of the true rationale of telomeres and telomerase regulation, and the potential applications of this knowledge in medicine, biodiversity conservation, further research on the diversity of telomerase in eukaryotic phylogeny should be carried out.

Acknowledgements

JTZ performed most of the research and data analyses and helped draft the manuscript. KSH and JQZ analysed and interpreted the raw data. All authors read and approved the final manuscript.

Funding

This work was supported by the Bethune Research Plan of Jilin University (grant number: 2020-31), the Training plan for Lixin excellent young teachers of Jilin University (grant number: 2021), the Foundation of Jilin Provincial Department of Education (grant number: JJKH20201026KJ) a Science and Technology Development Plan of Jilin Province (grant number: 20200202046YY).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Malik, A.S., Boyko, O., Atkar, N. and Young, W.F. (2001) A Comparative Study of MR Imaging Profile of Titanium Pedicle Screws. *Acta Radiologica*, **42**, 291-293. https://doi.org/10.1080/028418501127346846

[2] Greider, C.W. and Blackburn, E.H. (1987) The Telomere Terminal Transferase of Tetrahymena Is a Ribonucleoprotein Enzyme with Two Kinds of Primer Specificity. *Cell*, **51**, 887-898. https://doi.org/10.1016/0092-8674(87)90576-9

[3] Procházková Schrumpfová, P., Schorová, Š. and Fajkus, J. (2016) Telomere- and Telomerase-Associated Proteins and Their Functions in the Plant Cell. *Front Plant Sci.*, **7**, 851. https://doi.org/10.3389/fpls.2016.00851

[4] Kim, S., Parrinello, S., Kim, J. and Campisi, J. (2003) Mus Musculus and Mus Spretus Homologues of the Human Telomere-Associated Protein TIN2. *Genomics*, **81**, 422-432. https://doi.org/10.1016/S0888-7543(02)00033-2

[5] De Lange, T. (2015) A Loopy View of Telomere Evolution. *Front. Genet.*, **6**, 321. https://doi.org/10.3389/fgene.2015.00321

[6] Koonin, E.V. (2006) The Origin of Introns and Their Role in Eukaryogenesis: A Compromise Solution to the Introns-Early versus Introns-Late Debate? *Biol. Direct*, **1**, 22.

[7] Lambowitz, A.M. and Zimmerly, S. (2011) Group II Introns: Mobile Ribozymes That Invade DNA. *Cold Spring Harbor Perspect. Biol.*, **3**, a003616. https://doi.org/10.1101/cshperspect.a003616

[8] Jacobs, S.A., Podell, E.R. and Cech, T.R. (2006) Crystal Structure of the Essential N-Terminal Domain of Telomerase Reverse Transcriptase. *Nat Struct Mol Biol*, **3**, 218-225. https://doi.org/10.1038/nsmb1054

[9] Rouda, S. and Skordalakes, E. (2007) Structure of the RNA-Binding Domain of Telomerase: Implications for RNA Recognition and Binding. *Structure*, **15**, 1403-1412. https://doi.org/10.1016/j.str.2007.09.007

[10] Gillis, A.J., Schuller, A.P. and Skordalakes, E. (2008) Structure of the Tribolium Castaneum Telomerase Catalytic Subunit TERT. *Nature*, **455**, 633-637. https://doi.org/10.1038/nature07283

[11] Mitchell, M., Gillis, A., Futahashi, M., Fujiwara, H. and Skordalakes, E. (2010) Structural Basis for Telomerase Catalytic Subunit TERT Binding to RNA Template and Telomeric DNA. *Nat Struct Mol Biol*, **4**, 513-518. https://doi.org/10.1038/nsmb.1777

[12] Shcherbakova, D.M., Zvereva, M.E., Shpanchenko, O.V. and Dontsova, O.A. (2006) Telomerase: Structure and Properties of the Enzyme, Characteristics of the Yeast Telomerase. *Mol. Biol.* (*Moscow*), **40**, 1-15. https://doi.org/10.1134/S0026893306040042

[13] Osterhage, J.L., Talley, J.M. and Friedman, K.L. (2006) Proteasome-Dependent Degradation of Est1p Regulates the Cell Cycle-Restricted Assembly of Telomerase in Saccharomyces cerevisiae. *Nat. Struct. Mol. Biol.*, **13**, 720-728. https://doi.org/10.1038/nsmb1125

[14] Hsu, M., Yu, E.Y., Singh, S.M. and Lue, N.F. (2007) Mutual Dependence of Candida albicans Est1p and Est3p in Telomerase Assembly and Activation. *Eukaryot. Cell*, **6**, 1330-1338. https://doi.org/10.1128/EC.00069-07

[15] Collins, K. (2006) The Biogenesis and Regulation of Telomerase Holoenzymes. *Nat. Rev. Mol. Cell Biol.*, **7**, 484-494. https://doi.org/10.1038/nrm1961

[16] Denchi, E.L. (2009) Give Me a Break: How Telomeres Suppress the DNA Damage Response. *DNA Repair* (*Amst*), **8**, 1118-1126. https://doi.org/10.1016/j.dnarep.2009.04.013

[17] Meyne, J., Ratliff, R. and Moyzis, R.K. (1989) Conservation of the Human Telomere Sequence (TTAGGG)n among Vertebrates. *Proc Natl Acad Sci USA*, **86**, 7049-7053. https://doi.org/10.1073/pnas.86.18.7049

[18] Lejnine, S., Makarov, V.L. and Langmore, J.P. (1995) Conserved Nucleoprotein Structure at the Ends of Vertebrate and Invertebrate Chromosomes. *Proc Natl Acad Sci USA*, **92**, 2393-2397.

[19] Wu, R.A., Upton, H.E., Vogan, J.M. and Collins, K. (2017) Telomerase Mechanism of Telomere Synthesis. *Annu. Rev. Biochem.*, **86**, 439-460. https://doi.org/10.1073/pnas.92.6.2393

[20] Autexier, C. and Lue, N.F. (2006) The Structure and Function of Telomerase Reverse Transcriptase. *Annu. Rev. Biochem.*, **75**, 493-517. https://doi.org/10.1146/annurev-biochem-061516-045019

[21] Lingner, J., Hughes, T.R., Shevchenko, A., Mann, M., Lundblad, V. and Cech, T.R. (1997) Reverse Transcriptase Motifs in the Catalytic Subunit of Telomerase. *Science*, **276**, 561-567. https://doi.org/10.1146/annurev.biochem.75.103004.142412

[22] Friedman, K.L., Heit, J.J., Long, D.M. and Cech, T.R. (2003) N-Terminal Domain of Yeast Telomerase Reverse Transcriptase: Recruitment of Est3p to the Telomerase Complex. *Mol. Biol. Cell*, **14**, 1-13. https://doi.org/10.1126/science.276.5312.561

[23] Xia, J., Peng, Y., Mian, I.S. and Lue, N.F. (2000) Identification of Functionally Important Domains in the N-Terminal Region of Telomerase Reverse Transcriptase *Mol. Cell. Biol.*, **20**, 5196-5207. https://doi.org/10.1091/mbc.e02-06-0327

[24] Bosoy, D., Peng, Y., Mian, I.S. and Lue, N.F. (2003) Conserved N-Terminal Motifs of Telomerase Reverse Transcriptase Required for Ribonucleoprotein Assembly *in Vivo*. J. *Biol. Chem.*, **278**, 3882-3890. https://doi.org/10.1128/MCB.20.14.5196-5207.2000

[25] Friedman, K.L. and Cech, T.R. (1999) Essential Functions of Amino-Terminal Domains in the Yeast Telomerase Catalytic Subunit Revealed by Selection for Viable Mutants. *Genes Dev.*, **13**, 2863-2874. https://doi.org/10.1101/gad.13.21.2863

[26] Lue, N.F. and Li, Z. (2007) Modeling and Structure Function Analysis of the Putative Anchor Site of Yeast Telomerase. *Nucleic Acids Res.*, **35**, 5213-5222. https://doi.org/10.1093/nar/gkm531

[27] Romi, E., Baran, N., Gantman, M., Shmoish, M., Min, B., Collins, K. and Manor, H. (2007) High-Resolution Physical and Functional Mapping of the Template Adjacent DNA Binding Site in Catalytically Active Telomerase. *Proc. Natl. Acad. Sci. USA*, **104**, 8791-8796. https://doi.org/10.1073/pnas.0703157104

[28] Moriarty, T.J., Ward, R.J., Taboski, M.A. and Autexier, C. (2005) Disrupts Telomere Length Maintenance and Cellular Immortalization. *Mol. Biol. Cell*, **16**, 3152-3161. https://doi.org/10.1091/mbc.e05-02-0148

[29] Lue, N.F. and Peng, Y. (1998) Negative Regulation of Yeast Telomerase Activity through an Interaction with an Upstream Region of the DNA Primer. *Nucleic Acids Res.*, **26**, 1487-1494. https://doi.org/10.1093/nar/26.6.1487

[30] Egan, E.D. and Collins, K. (2012) Biogenesis of Telomerase ribonucleoproteins. *RNA*, **18**, 1747-1759. https://doi.org/10.1261/rna.034629.112

[31] Schmidt, J.C. and Cech, T.R. (2015) Human Telomerase: Biogenesis, Traffificking, Recruitment, and Activation. *Genes Dev.*, **29**, 1095-1105. https://doi.org/10.1101/gad.263863.115

[32] Alves, D., Li, H., Codrington, R., Orte, A., Ren, X., Klenerman, D. and Balasubramanian, S. (2008) Single-Molecule Analysis of Human Telomerase Monomer. *Nat. Chem. Biol.*, **4**, 287-289. https://doi.org/10.1038/nchembio.82

[33] Lai, C.K., Mitchell, J.R. and Collins, K. (2001) RNA Binding Domain of Telomerase Reverse Transcriptase. *Mol. Cell. Biol.*, **21**, 990-1000. https://doi.org/10.1128/MCB.21.4.990-1000.2001

[34] Zaug, A.J., Podell, E.R. and Cech, T.R. (2008) Mutation in TERT Separates Processivity from Anchor-Site Function. *Nat Struct Mol Biol.*, **8**, 870-872. https://doi.org/10.1038/nsmb.1462

[35] Meier, B., Clejan, I., Liu, Y., Lowden, M., Gartner, A., Hodgkin, J. and Ahmed, S. (2006) Trt-1 Is the Caenorhabditis elegans Catalytic Subunit of Telomerase. *PLOS Genet*, **2**, 18. https://doi.org/10.1371/journal.pgen.0020018

[36] Xie, M., Podlevsky, J.D., Qi, X., Bley, C.J. and Chen, J.J. (2010) A Novel Motif in Telomerase Reverse Transcriptase Regulates Telomere Repeat Addition Rate and Processivity. *Nucleic Acids Res*, **6**, 1982-1996. https://doi.org/10.1093/nar/gkp1198

[37] Bernardes de Jesus, B. and Blasco, M.A. (2013) Telomerase at the Intersection of Cancer and Aging. *Trends in Genetics*, **9**, 513-520. https://doi.org/10.1016/j.tig.2013.06.007

[38] Calado, R.T. and Young, N.S. (2009) Telomere Diseases. *N. Engl. J. Med.*, **24**, 2353-2365. https://doi.org/10.1056/NEJMra0903373

[39] Singh, M., Wang, Z., Koo, B.K., Patel, A., Cascio, D., Collins, K., *et al.* (2012) Structural Basis for Telomerase RNA Recognition and RNP Assembly by the Holoenzyme La Family Protein p65. *Mol Cell*, **47**, 16-26. https://doi.org/10.1016/j.molcel.2012.05.018

[40] Jansson, L.I., Akiyama, B.M., Ooms, A., Lu, C., Rubin, S.M. and Stone, M.D. (2015) Structural Basis of Template-Boundary Definition in Tetrahymena Telomerase. *Nat Struct Mol Biol*, **22**, 883-888. https://doi.org/10.1038/nsmb.3101

[41] Cash, D.D. and Feigon, J. (2017) Structure and Folding of the Tetrahymena Telomerase RNA Pseudoknot. *Nucleic Acids Res*, **45**, 482-495. https://doi.org/10.1093/nar/gkw1153

[42] Lai, C.K., Miller, M.C. and Collins, K. (2002) Template Boundary Definition in Tetrahymena Telomerase. *Genes Dev*, **16**, 415-420. https://doi.org/10.1101/gad.962602

[43] Miller, M.C., Liu, J.K. and Collins, K. (2000) Template Definition by Tetrahymena Telomerase Reverse Transcriptase. *EMBOJ*, **19**, 4412-4422. https://doi.org/10.1093/emboj/19.16.4412

[44] Mason, D.X., Goneska, E. and Greider, C.W. (2003) Stem-Loop IV of Tetrahymena Telomerase RNA Stimulates Processivity in Trans. *Mol Cell Biol*, **23**, 5606-5613. https://doi.org/10.1128/MCB.23.16.5606-5613.2003

[45] Sharon, E., Freeman, R., Riskin, M., Gil, N., Tzfati, Y. and Willner, I. (2010) Optical, Electrical and Surface Plasmon Resonance Methods for Detecting Telomerase Activity. *Anal. Chem.*, **82**, 8390-8397. https://doi.org/10.1021/ac101976t

[46] Cohen, S.B., Graham, M.E., Lovrecz, G.O., Bache, N., Robinson, P.J. and Reddel, R.R. (2007) Protein Composition of Catalytically Active Human Telomerase from Immortal Cells. *Science*, **315**, 1850-1853. https://doi.org/10.1126/science.1138596

[47] Skvortsov, D.A., Zvereva, M.E., Shpanchenko, O.V. and Dontsova, O.A. (2011) Assays for Detection of Telomerase Activity. *Acta Naturae*, **1**, 48-68. https://doi.org/10.32607/20758251-2011-3-1-48-68

[48] Cohen, S.B. and Reddel, R.R. (2008) A Sensitive Direct Human Telomerase Activity Assay. *Nat. Meth.*, **5**, 355-360. https://doi.org/10.1038/nmeth.f.209