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Mechanisms of Ageing

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Introduction

The objective of this study was to further understand the mechanism of ageing and to see what roles particular enzymes may play therein.

The key enzymes studied in the project were sirtuins and telomerase. Sirtuins are a class of proteins that can act as mono-ribosyltransferase or deacylase. There are seven different subunits of sirtuins involved in humans. By analyzing the different functions of each subunit and discovering how they mechanically or structurally interact with each other at the molecular level, their function in ageing can be discerned.

Telomerase adds repeats to the ends of DNA strand to ensure that they do not degenerate or decrease in length. There are many subunits of telomerase, each with its own function which is responsible for the overall addition of repeats to the telomere regions of chromosome ends. The research performed also involved investigating what particular subunits were involved and in which specific role.

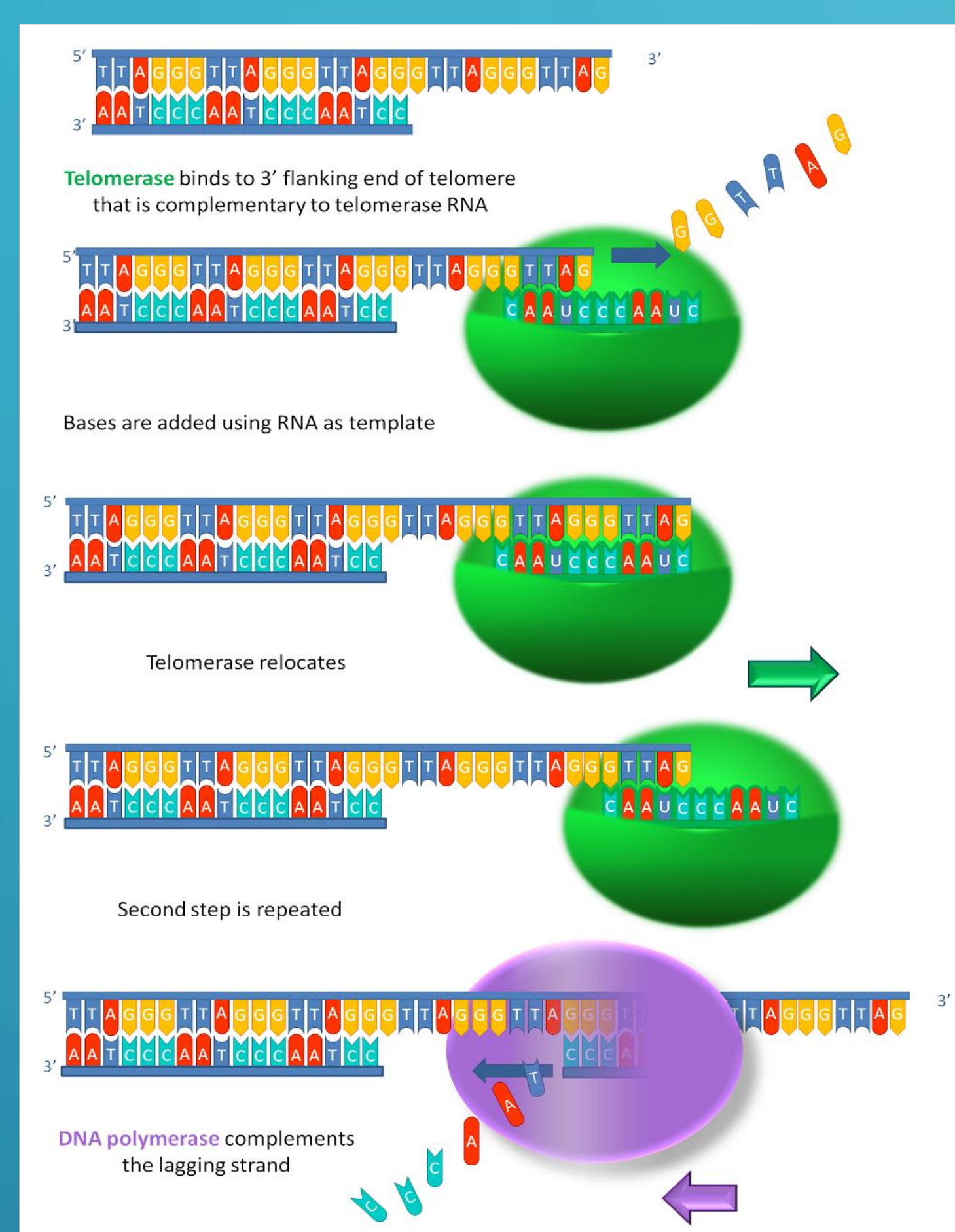


Figure 1. Graphical Illustration of Telomerase adding nucleotides to the ends of chromosomes.

Through macro-molecular modelling simulations, disturbing or enhancing the interactions of each of the subunits we can predict which alterations may cause apoptosis or continued normal cell activity. To do this, each subunit was analyzed at the molecular level to see what types of regions were present and what role they played in the overall function of each subunit.

Methodology

The NCBI database GenBank was used to analyze the DNA sequences as well as the protein sequences for each subunit of telomerase and sirtuins. GenBank displayed the important regions in each subunit which provided us with the information regarding interactions of that specific subunit with the other protein moieties. Other regions of their respective gene sequences that were analyzed were the promoter region, 5' Untranslated Region (UTR) and the 3' UTR.

The sequences of the subunits from humans were also compared with other organisms by running a BLAST. This program allows the two sequences to be aligned (i.e. compared side by side, base per base) and to highlight any nucleotide differences.

Results

The telomerase protein had at least five subunits responsible for its function. They include Telomerase Reverse Transcriptase (TERT), TERC, TEP1, DCK1 and the Cajal Body

TERT is the major subunit. It has 15 introns, 16 exons, multiple binding regions and a promoter region that is GC rich but lacks a TATA and CAAT box. The TERT gene has three phosphorylation sites, four motif sites and two RNA-interacting domains. The motif sites include GQ, CP, QFP and T amino acid residues.

TERC is a RNA gene that encodes the template that is used for the telomere region. DCK1 is a SnoRNP protein which has seven phosphorylation sites, two Nuclear localization regions and an acetylation site.

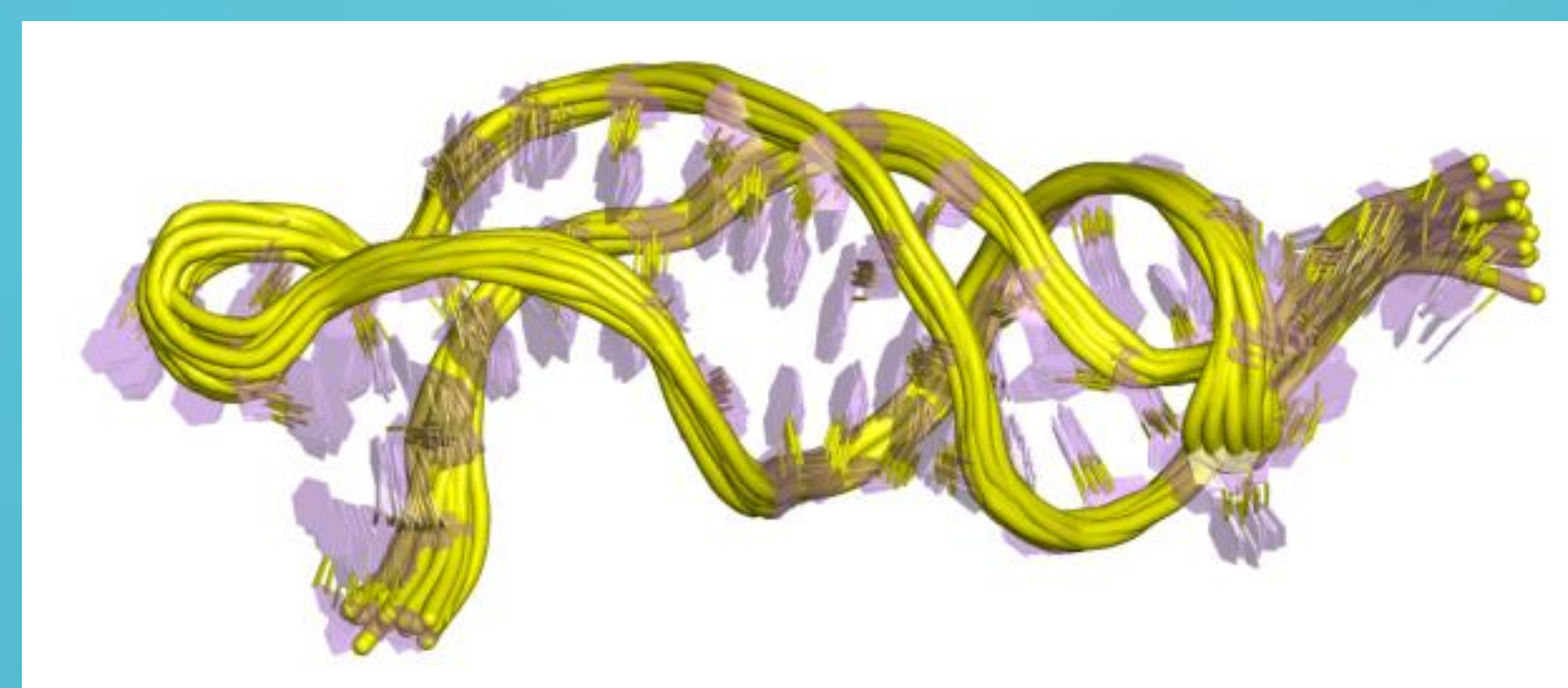


Figure 2. 3-D rendition of the Telomerase RNA Component (TERC).

TEP1 is the enzyme that facilitates the addition of telomeres to the ends of chromosomes. This sub-unit is especially interesting because it has four N-terminal regions as well as 21 WD-40 repeats (small 40 amino acid motifs). These repeated sequences act as a site for protein-protein interactions and to serve as platforms for the assembly of protein complexes. It also consists of three other regions: TROVE, NACHT and DUF4062. The latter one has an unknown function. The TROVE domain is made up of 300-500 residues and may be involved in binding RNA components of ribonucleoprotein. NACHT is made up of 300-400 amino acid residues and contains seven conserved motifs.

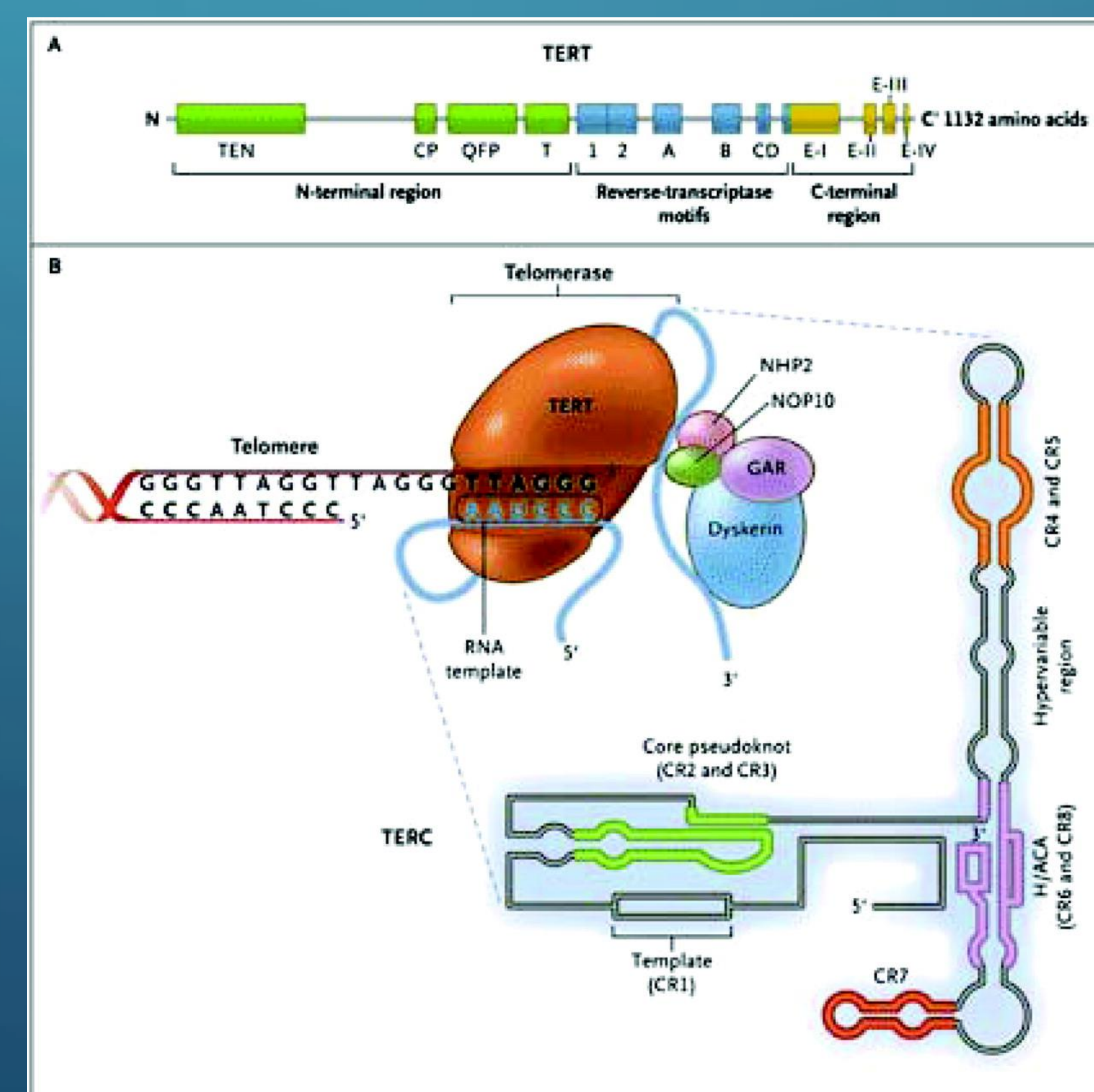


Figure 3. Conceptual diagram showing the interactions of the different subunits of Telomerase.

Results

Cajal Body protein is 548 amino acid residue long and has many phosphorylation sites. This protein encodes for a ribonucleoprotein complex which is an essential component of telomerase holoenzyme complex. This enzyme has Cajal bodies which are sub-organelles that have been shown to assist in the transport of telomerase to telomeres and it also contributes to the biogenesis of the telomerase enzyme.

Sirtuin	Location	Interactions	Biology
SIRT 1	Nucleus	FOXO, PGC-1 α , NF- κ B, Ku70	Metabolism, stress
SIRT 2	Cytosol	Tubulin, H4, FOXO	Cell cycle
SIRT 3	Mitochondria	AceCS2, GDH	Thermogenesis, ATP Production
SIRT 4	Mitochondria	GDH, IDE, ANT	Insulin secretion
SIRT 5	Mitochondria	CPS1	Urea cycle
SIRT 6	Nucleus	Histone H3, NF- κ B	Base excision repair, metabolism
SIRT 7	Nucleolus	Pol I	rDNA transcription

Table 1. Comparison of the seven subunits of sirtuins in Homo sapiens including their location within the cell, interactions and their biological impact.

There are seven sirtuins subunits which have very similar sequences. They all have binding sites for NAD⁺, Zn and other substrates.

PREDICTED: NAD-dependent protein deacetylase sirtuin-1 isoform X1 [Homo sapiens]
Sequence ID: ref|XP_005269718.1| Length: 708 Number of Matches: 1

Score	Expect	Method	Identities	Positives	Gaps
934 bits(2414)	0.0	Compositional matrix adjust.	452/452(100%)	452/452(100%)	0/452(0%)
Query 1	MFDEYFRKDRPFFKFAKEIYFGQFPLSCHKFKIALSDKEGKLLRNVTQINDILEQVAG				60
Sbjct 257	MFDEYFRKDRPFFKFAKEIYFGQFPLSCHKFKIALSDKEGKLLRNVTQINDILEQVAG				316
Query 61	IQRLLQCHGSFATASCLICKYKVDCEAVRGDIFNQVPRCPCFADEPLAMKPEIVVFG				120
Sbjct 317	IQRLLQCHGSFATASCLICKYKVDCEAVRGDIFNQVPRCPCFADEPLAMKPEIVVFG				376
Query 121	ENLPEQFHRAMKYDKDEVLLIVIGSSSLKVRVVALIPSSIPHEVQILINREPLFLHFD				180
Sbjct 377	ENLPEQFHRAMKYDKDEVLLIVIGSSSLKVRVVALIPSSIPHEVQILINREPLFLHFD				436
Query 181	VLLGDQCVIINELCHRLGGVAKLCCIFVPLSEITEKFPRTQELAYLSELPFTELHVS				240
Sbjct 437	VLLGDQCVIINELCHRLGGVAKLCCIFVPLSEITEKFPRTQELAYLSELPFTELHVS				496
Query 241	EDSSSPERTSPDSSSVITVLLDQAANKNDLIVSSEKGCMEKEQEVQSRNVESTAQM				300
Sbjct 497	EDSSSPERTSPDSSSVITVLLDQAANKNDLIVSSEKGCMEKEQEVQSRNVESTAQM				556
Query 301	ENFDLQVSSSTGKNERISVAGTVRKCWENRVAKEQISRLLDGNQVLFLEPNRYIFHGA				360
Sbjct 557	ENFDLQVSSSTGKNERISVAGTVRKCWENRVAKEQISRLLDGNQVLFLEPNRYIFHGA				616
Query 361	EVYSDSDVLLSSSSCGSNSDSGTCQSPSLEEFMEDESEIEEFYNGLEDFDVPERAGGA				420
Sbjct 617	EVYSDSDVLLSSSSCGSNSDSGTCQSPSLEEFMEDESEIEEFYNGLEDFDVPERAGGA				676
Query 421	FGFDGDDQEAINEAISVQVEVIMDMYFNSKIS		452		
Sbjct 677	FGFDGDDQEAINEAISVQVEVIMDMYFNSKIS		708		

Figure 4. BLAST comparing two of the subunits of sirtuin. Aligned are SIRT1 and SIRT2.

Conclusion

There are regions within the DNA sequence that can bind specific repair and/or maintenance macro-molecules which allows them to function in preserving chromosome integrity. By disturbing, inhibiting or enhancing sirtuin and telomerase function, the rate at which a cell dies can be manipulated.

Acknowledgements

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References

Bernardes De Jesus, Bruno, et al. "Telomerase Gene Therapy in Adult and Old Mice Delays Aging and Increases Longevity without Increasing Cancer." EMBO MOLECULAR MEDICINE 4.8 (2012): 691-704. WILEY-BLACKWELL. Web. 7 Oct. 2013.
Kitada, Munehiro, et al. "Sirtuins and Renal Diseases: Relationship with Aging and Diabetic Nephropathy." CLINICAL SCIENCE 124.3-4 (2013): 153-54. Clinical Science. PORTLAND PRESS LTD, 5 Oct. 2012. Web. 11 Nov. 2013.
Li, Ying, et al. "Telomerase and Aging." Proc. of 2012 International Conference on Education Reform and Management Innovation, Peoples R China, Shenzhen. INFORMATION ENGINEERING RESEARCH INST, 2013. Web. 28 Oct. 2013.