Review Article

Comparison of Telomere Structure in Eukaryotes

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ABSTRACT

Telomeres are DNA-protein complexes that are located at the ends of eukaryotic chromosomes. The fusion of broken chromosome ends is prevented by the presence of telomeres, which act to inhibit this process. This specific function of telomeres serves to distinguish normal chromosome ends from double-stranded breaks in DNA. Telomeres contain a series of short, repeated sequences arranged in a tandem array. The number of repeats varies between different organisms, with a range of 20 to 1,000 repeats being typical. A G-rich strand is replicated by lagging strand synthesis, which creates a 3' overhang. In addition, a complementary C-rich strand is replicated by leading strand synthesis. The objective of this study is to undertake a comparative analysis of the structure of telomeres in Saccharomyces cerevisiae, Saccharomyces pombe and mammals. In Saccharomyces cerevisiae, the Rap1 protein binds to the double-stranded telomeric sequences, as well as to the Rif1 and Rif2 proteins, which regulate telomere length. Cdc13 and the Cdc13-interacting factors Ten1 and Stn1 bind to the single-stranded overhang. In Saccharomyces pombe telomeres, Taz1 binds to the double-stranded DNA (dsDNA), and Rap1 and Rif1 also bind to the ds region via Taz1. Pot1 interacts with Tpz1, forming a complex that binds to the 3' overhang. The protein Poz1 serves to connect the dsDNA binding complex, comprising Taz1 and Rap1, to the ssDNA binding complex, which includes Pot1 and Tpz1. Furthermore, Ccq1 interacts with Tpz1 and facilitates the recruitment of telomerase. The Stn1/Ten1 complex exhibits a binding affinity for a single-stranded telomere. In mammalian telomeres, the shelterin complex that binds double-stranded telomeric DNA is composed of six subunits. The double-stranded telomeric DNA is bound by TRF1 and TRF2. TPP1 and POT1 are capable of binding single-stranded DNA. TIN2 serves to connect the dsDNA binding complex TRF1/TRF2 to the ssDNA binding complex POT1/TPP1. Rap1 binds to the telomere by interacting with TRF1 and TRF2. Moreover, this study will address the regulation and comparison of the shelterin complex. Additionally, in mammals, the activation of DNA damage response pathways is necessary when double-strand DNA is broken. This, in turn, elucidates the specific repair pathways that are employed. We conclude by discussing the T-loop structure, as telomeres in several species have been shown to fold back into a structure called a T-loop, which is believed to mediate telomere protection.

Keywords: Telomere, Shelterin, T-Loop Structure.

1. Introduction

1.1. Telomere Biology

Telomeres are DNA-protein complexes that are located at the ends of eukaryotic chromosomes. The fusion of broken chromosome ends is prevented by the presence of telomeres, which act as caps on chromosomes. This specific function of telomeres serves to distinguish normal chromosome ends from double-stranded breaks in DNA (8). Telomeres contain a series of repeated sequences, which are arranged in a tandem array (6). The number of repeats varies between different organisms, with a range of 20 to 1,000 repeats being typical. A G-rich strand is replicated by lagging strand synthesis, resulting in the formation of a 3' overhang. In contrast, a complementary C-rich strand is replicated by leading strand synthesis (43). The ends of linear eukaryotic chromosomes encounter significant challenges during the process of cellular DNA replication. This issue is referred to as end replication, which arises from the fact that the chromosomes are replicated by DNA polymerases that are only capable of synthesising DNA in the 5' to 3' direction. A complementary DNA strand is synthesised continuously only along the leading strand; however, end replication problems are encountered at the 5' end of the lagging strand. This is due to the fact that RNA primers, which are synthesised by DNA polymerase α/primase, serve to prime the synthesis of Okazaki fragments in the lagging strand. The RNA primers must then be removed and replaced with DNA by DNA polymerase δ. However, DNA polymerase δ is unable to synthesise DNA at the lagging strand's 5' end, resulting in the formation of a gap at this site and a 3' single-stranded overhang. If this issue were to manifest in each replication cycle, it would result in a reduction in chromosome length with each cell division (Figure 1) (6, 8, 27). The issue of end replication can be addressed by the RNA-protein enzyme telomerase, which is responsible for creating the terminal sequence at the 3' end through a process of reverse transcription using its own RNA template. This results in the elongation of the parental strand by telomerase, followed by the completion of the lagging strand by the action of DNA polymerase machinery (9). Consequently, telomerase is essential for combating the gradual degradation of chromosome ends and for ensuring the maintenance of optimal telomere length (39). An additional issue associated with chromosome ends, also referred to as the end-protection problem, arises due to the fact that cells possess mechanisms capable of identifying chromosome ends as double-strand breaks. This subsequently triggers the initiation of DNA repair processes, which can potentially lead to detrimental consequences for genomic integrity (12). To circumvent the end protection issue, a capping protein such as Pot1 is recruited to chromosome ends in fission yeast and in higher eukaryotes (3). Furthermore, a specific DNA structure may be formed; this is referred to as the t-loop, whereby a 3' overhang invades an array of DNA repeats (20). As a consequence of this protective mechanism, DNA repair processes, such as non-homologous end joining (NHEJ) and homologous recombination (HR), can be impeded by telomeres (16). As shown Figure 2, the telomere sequence is highly conserved in different groups of organisms.

2. Results

2.1. Structure of the Saccharomyces Cerevisiae Telomere

The sequence of Saccharomyces cerevisiae telomeric DNA is constituted by TG1-3/CA1-3 repeats, which are followed by a G-rich overhang at the 3' end of the chromosome (36). Protein complexes have been observed to bind to telomeric sequences that are involved in a number of different processes, including telomere maintenance, telomere length regulation, protection of the G-rich overhang, transcriptional repression and nuclear positioning (19). The regulation of telomerase is essential for the maintenance of telomere length in each organism. In the budding yeast, a number of positive regulators are necessary for the activity of telomerase at the telomeres. These positive regulators include the following: cdc13, which binds to Est1 (telomerase subunit); the Ku heterodimer; and the Tel1 kinase. Additionally, there are negative regulators that impede telomerase activity at the telomeres, including the Rif1 and Rif2 proteins and the Pif1 helicase (5, 18). Telomeric sequences in budding yeast have been observed to bind directly to the Myb domain of the Rap1 protein, which has been demonstrated to function in the inhibition of telomerase recruitment (38). The negative regulators of telomerase, Rif1 and Rif2, have been observed to interact with Rap1. Therefore, telomeres can be elongated by the deletion of either Rif1 or Rif2 (42). A comparable phenotype can also be observed in the context of Rap1 deletion. In addition, telomerase has been observed to bind to Est1, an important regulatory subunit that interacts with TLC1 and Est3 in budding yeast. The Cdc13 protein interacts with the telomerase subunit Est1, thereby facilitating the recruitment of telomerase to telomere ends and resulting in telomere elongation. Cdc13 contains OB fold domains (Figure 3) (38). The presence of G-rich single-stranded DNA is indispensable for the functioning of telomerase. Cdc13 interacts directly with the Ten1 and Stn1 proteins, thereby forming the CST protein complex. The CST proteins bind to the 3' overhang, which plays a regulatory role in telomerase activity at chromosome ends. Consequently, the CST complex is involved in both telomere protection and the inhibition of telomerase action (Fig. 3) (2). A number of proteins have been identified as binding to telomeres, including the MRX complex, Tel1, and the Ku protein. These proteins have been shown to play an essential role in both the DNA repair pathway and the DNA damage checkpoint (38). Cdc13 is phosphorylated by Cdk1. Phosphorylated Cdc13 plays a positive role in telomerase recruitment by interacting with Est1, which results in telomere elongation. Subsequently, the function of Cdc13 is altered through its interaction with Stn1 and Ten1, which can inhibit telomerase activity. Furthermore,

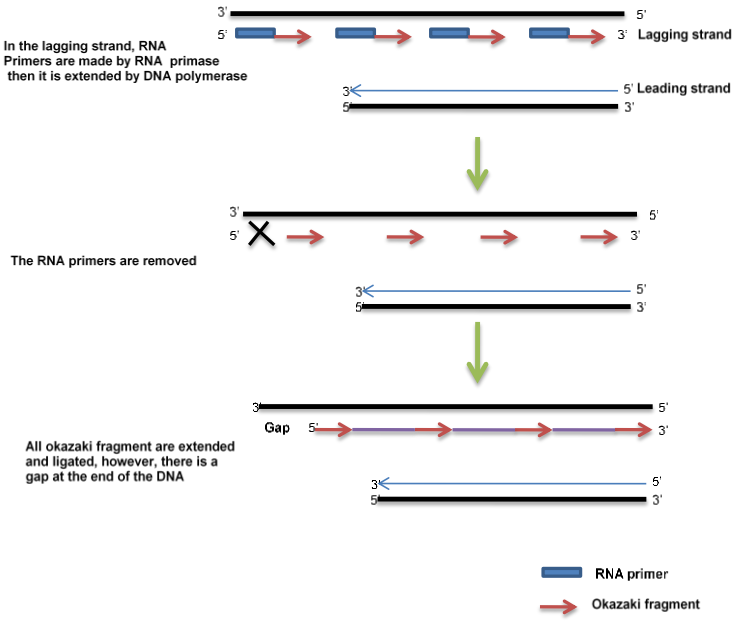

Figure1. The end replication problem. Lagging strand DNA synthesis does not complete the replication of the 5' end resulting in a 3' overhang. Leading strand DNA synthesis, however, complete the replication of the 5' end resulting in a blunt end.

Occurrence	Consensus telomere repeat sequence
Saccharomyces cerevisiae	TG1-3
Schizosaccharomyces pombe	TTACAG1-8
Neurospora crassa	TTAGGG
Paramecium	TTGGGG
Trypanosoma	TAGGGG
Chlamydomonas	TTTTAGGG
Arabidopsis	TTTAGGG
Nematodes	TTAGGC
Vertebrates	TTAGGG

Figure 2. Evolutionary Conservation of Telomeric repeat sequences. Telomeric repeat sequences and also telomere function are conserved throughout eukaryotes.

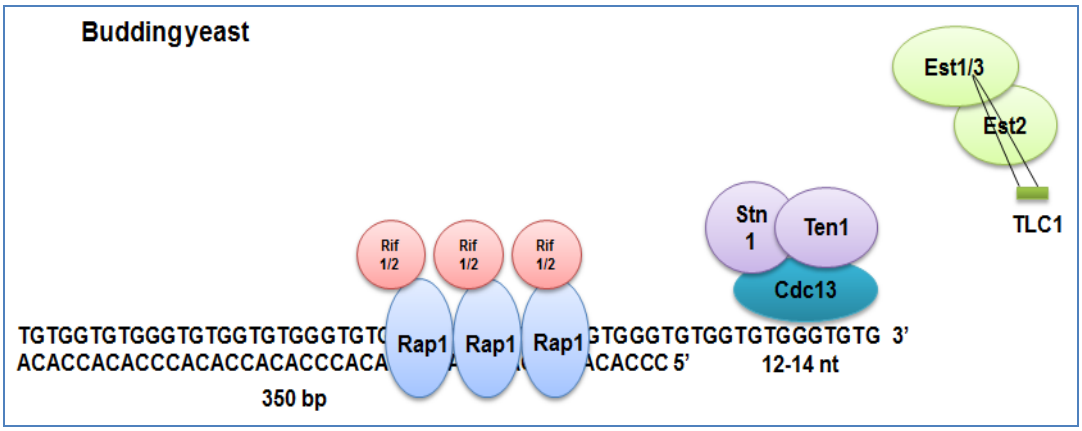

Figure 3. Structure of *Saccharomyces cerevisiae* telomeres. Rap1 binds the double-stranded telomeric sequences and also binds Rif1 and Rif2 which regulate telomere length. Cdc13 and the Cdc13-interacting factors Ten1 and Stn1 bind to the single strand overhang.

the DNA polymerase α/primase complex interacts with the CST complex, resulting in the inhibition of telomere elongation by the CST protein (28). The Tel1 protein is responsible for the recruitment of telomerase and subsequent elongation of the telomere in instances where the telomere has been shortened (5). As illustrated in the following diagram, there is a competition between Rif2 and Tel1 to bind to the Xrs2 component of the MRX complex. This results in Rif1 and Rif2 inhibiting the function of Tel1, but not the MRX complex, in its ability to bind to Xrs2. Moreover, it seems that Rap1 inhibits the interaction between MRX and Tel1 (Figure 4) (20).

2.2. Structure of the Schizosaccharomyces Pombe Telomere

The telomere of Schizosaccharomyces pombe exhibits a high level of conservation with the human telomere. In this organism, a protein known as Taz1 (its orthologue is TRF1/2 in mammals) is directly bound to telomere duplex DNA repeats through its Myb domain. Taz1 plays a crucial role in maintaining telomere length and also in inhibiting recombination (33). Taz1 plays a pivotal role in telomere protection and telomerase inhibition. Consequently, Taz1 deletion results in telomere elongation (10). Furthermore, Taz1 facilitates replication fork passage. Consequently, in the absence of Taz1, replication forks are restricted, leading to telomerase recruitment. Furthermore, telomeric resection is constrained by Taz1 (33). The protein Pot1 (protection of telomeres) plays a critical role in telomere function, serving both to protect telomeres and to regulate telomerase activity. In the absence of Pot1, telomeres are lost and can only survive by undergoing chromosome circulation (7). Taz1 has the capacity to recruit two proteins, namely Rap1 and Rif1. These, in turn, have the ability to exert a negative regulatory effect on telomerase activity (37). Taz1 and Rap1 are responsible for the inhibition of non-homologous end joining (NHEJ). The ability of Rap1 to bind to DNA is indirect, occurring via Taz1. Rif1 is localised in the telomere via Taz1 binding, which also recruits Poz1, which in turn binds to Tpz1. Tpz1 is involved in the interaction with both Ccq1, which plays a role in telomerase recruitment, telomere maintenance and checkpoint inhibition, and also Pot1, which interacts with single-stranded DNA. The Pot1/Tpz1 complex is linked to the Taz1/Rap1 complex via Poz1. In fission yeast, Stn1 and Ten1 interact with each other, yet do not interact with Pot1. They play a pivotal role in maintaining telomere stability. The Pot1, Tpz1, Stn1, and Ten1 proteins possess OB fold domains, which are oligonucleotide- or oligosaccharide-binding domains, and are utilised for binding to the 3' overhangs (14, 17). In fission yeast, the protein telomerase subunit is designated Trt1, while TER1 represents the RNA component. The function of TER1 is to facilitate the connection between Est1 and Trt1, as well as to permit the synthesis of telomere repeats (Figure 5) (28).

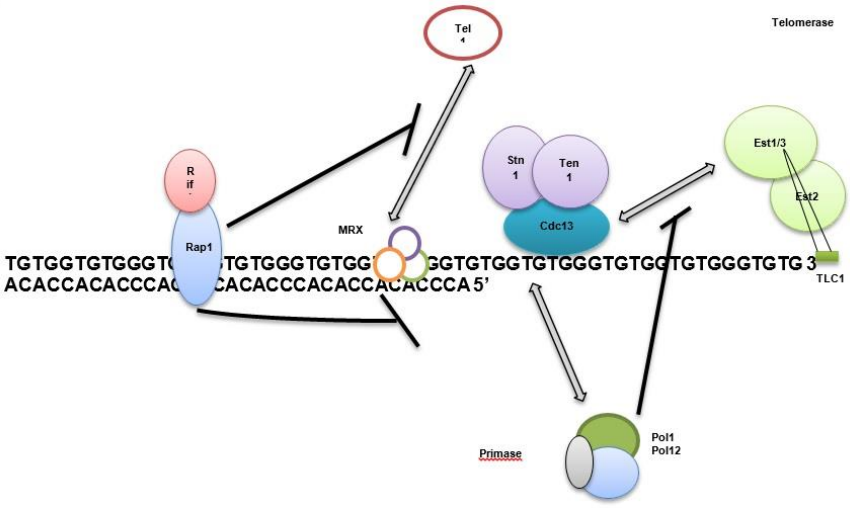

Figure 4. Summary of some of the proposed steps in TG tract length-dependent regulation of telomerase action. The Rap1-Rif complex creates a negative signal which blocks telomerase recruitment. Rif2 competes with Tel1 to bind to the Xrs2 component of MRX, hence it inhibits Tel1 binding. Cdc13 is phosphorylated by Cdk1, which creates a positive signal for telomerase recruitment by interacting with Est1 subunit. The DNA polymerase α /primase complex interacts with the CST complex, thereby inhibiting telomerase recruitment.

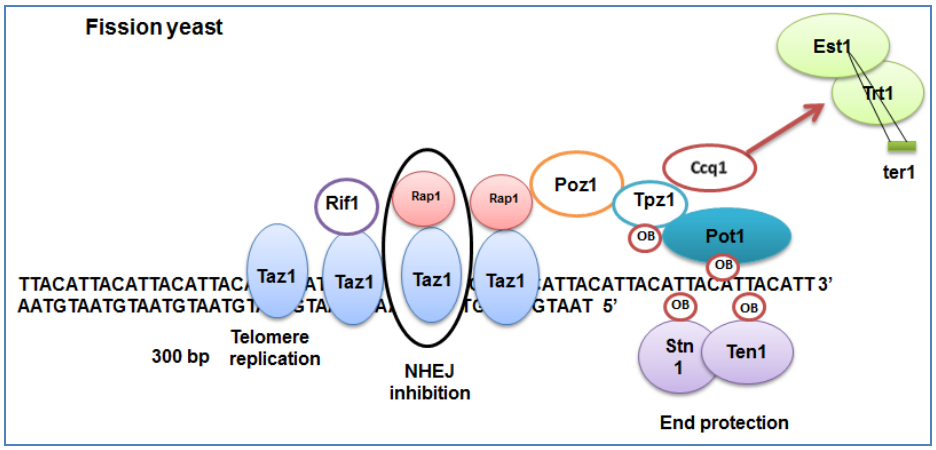

Figure 5. Structure of *Saccharomyces Pombe* Telomeres.Taz1 binds the dsDNA and also Rap1 and Rif1 bind to the ds region via Taz1. Pot1 interacts with Tpz1 and binds to the 3′ overhang. Poz1 connects the dsDNA binding complex Taz1/Rap1 to the ssDNA binding complex Pot1/Tpz1. Ccq1 also interacts with Tpz1, recruits telomerase. Stn1/Ten1 complex binds to single strand telomere.

The first telomere end-binding proteins were isolated from the ciliate known as TEBPα /TEBPβ, which bind to the single-stranded DNA, and contain OB fold domains. Pot1 in fission yeast is an orthologue of TEBPα in ciliates, and also both Pot1 and Tpz1 include OB fold domains. It can therefore be concluded that there is an evolutionary conservation between TEBPα /TEBPβ in ciliates and Pot1 in fission yeast (4).

2.3. Structure of the Mammalian Telomere

In mammals, telomeric DNA is constituted by a series of repeated sequences, comprising the sequence TTAGGG. Two Myb domain proteins, designated TRF1 and TRF2, were identified as binding to the telomere duplex DNA repeats. The function of TRF1 is to impede the recruitment of telomerase; thus, the overexpression of TRF1 in human cells results in telomere shortening. It has been demonstrated that Rap1 directly interacts with TRF2. However, a protein known as TIN2 has been shown to have a direct interaction with TRF1/TRF2. The TPP1/POT1 complex is connected to the TRF1/2 complex via TIN2. A complex of six telomere-associated proteins in mammals is referred to as shelterin (12). A comparable structure was also identified in fission yeast, in which TPZ1/POT1 is linked to TAZ1 via POZ1. In humans, POT1 has the capacity to bind to double-stranded DNA indirectly, while its binding to single-stranded DNA is direct (38). TPP1/POT1 binds to ssDNA and, like TPZ1/POT1 in fission yeast and Cdc13 in budding yeast, contains OB fold domains (Figure 6) (35). The equilibrium between telomere attrition and telomere lengthening is regulated by telomeric proteins. With the exception of Tpp1, which interacts with telomerase directly and recruits it to telomeres, all of the telomeric proteins (TRF1/2, TIN2, POT1, and RAP1) act as negative regulators of telomerase (35). The function of Pot1 as a negative regulator of telomerase is due to its binding to the 3' overhang (23). A competition exists between Pot1 and telomerase for binding to the 3' overhang. Consequently, when Pot1 binds to the 3' overhang, it prevents telomerase from binding to telomere ends (30). Furthermore, a reduction in TPP1 concentration results in a decline in Pot1 levels at the 3' overhang, which subsequently facilitates telomerase recruitment to telomeres. Consequently, telomeres can be elongated in the absence of TPP1 (26). Shelterin fulfils two distinct functions: firstly, it protects telomeres from DNA damage repair pathways, and secondly, it regulates telomere length by telomerase (24). As previously stated, all three organisms possess two distinct protein complexes. One type of protein complex is involved in the activation and recruitment of telomerase, while the other type of protein complex serves to negatively regulate telomerase recruitment.

2.4. Regulation of Telomerase by Shelterin

Human telomerase is a ribonucleoprotein complex comprising TERT (telomerase reverse transcriptase), which is capable of generating single-stranded DNA using single-stranded RNA as a template, TERC (telomerase RNA component) or TR (telomerase RNA component), and dyskerin (DKC1). The complex is situated within the nucleus in Cajal bodies and can be transferred to telomeres by the accessory factor TCAB1. The complex is initially in an immature form, which must be converted to the mature form by the action of Pontin (also known as RUVBL1) and Reptin (also known as RUVBL2). The mature conformation is then capable of adding telomeric repeats at chromosome ends (Figure 7) (41).

2.5. Comparison of Shelterin to Telomeric Proteins in other Eukaryotes

It has been demonstrated that certain telomeric proteins in mammals are conserved with their counterparts in fission yeast, specifically TRF1/2 and POT1. These proteins have been shown to be evolutionarily conserved in humans as orthologs of Taz1 and Pot1 in fission yeast, respectively. Similarly, Rap1 in fission yeast, which binds to Taz1, binds to TRF2 in mammals. With the exception of Rap1 in budding yeast, which is the sole telomeric protein that has been shown to be conserved with Rap1 in shelterin, none of the telomeric proteins in budding yeast have been identified

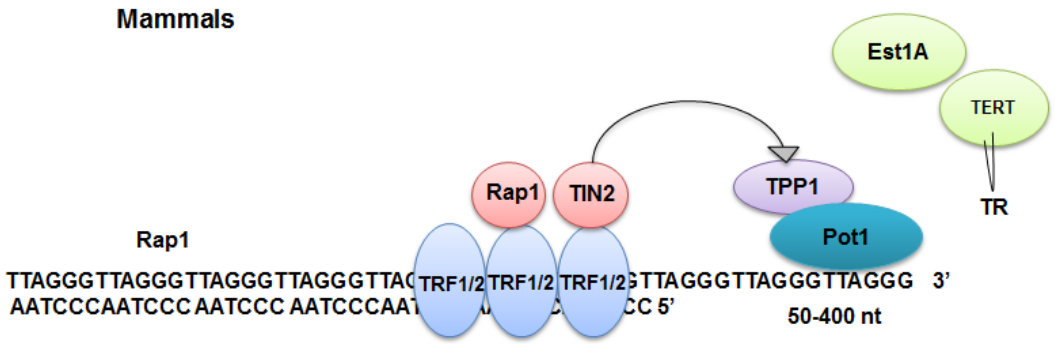

Figure 6. Structure of the mammalian Telomere. The shelterin complex bind double stranded-telomeric DNA consists of six subunits: TRF1 and TRF2 which bind directly the double-stranded telomeric DNA. TPP1 and POT1 bind single-stranded DNA.TIN2 connects the dsDNA binding complex TRF1/TRF2 to the ssDNA binding complex POT1/TPP1. Rap1 binds the telomere by interacting with TRF1 and TRF2.

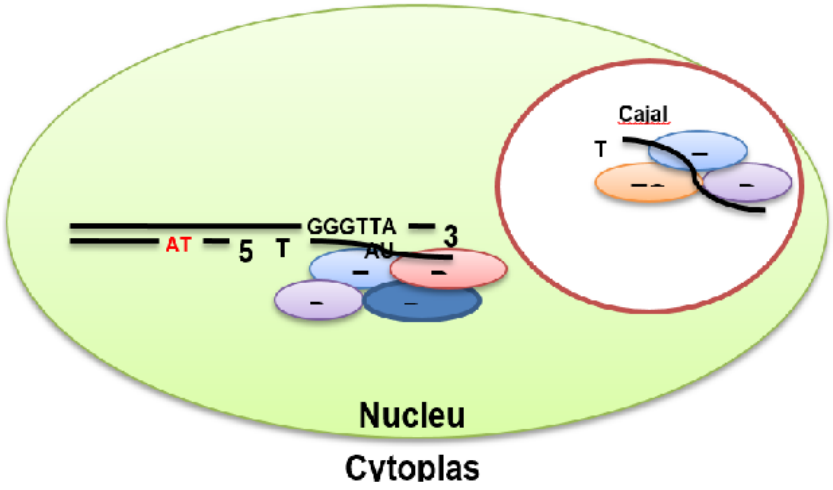

Figure 7. The telomerase complex. Telomerase is composed of TERT that provides reverse transcriptase activity to the complex and uses TERC, the RNA component of telomerase, as a template.

as being similar to shelterin. Additionally, TPP1/Pot1 in mammals is an orthologue of TEBPα/TEBPβ in ciliates. This suggests that TEBPα/TEBPβ is conserved in both fission yeast and mammals (Figure 8) (35). Telomeric single-stranded DNA is identified by TEBP in Oxytricha nova, Cdc13 in S. cerevisiae, and Pot1 in S. pombe, humans, and other higher eukaryotes due to the fact that these proteins bind to ssDNA through a conserved motif, namely the OB fold domain. It is noteworthy that Cdc13 is associated with Pot1 due to the shared structural characteristics of its OB-fold domain. Nevertheless, it remains uncertain whether Cdc13 and Pot1 are orthologous proteins, given the limited sequence similarity observed in their OB folds domain (Figure 8) (31).

2.6. Telomers Solve the End Protection Problem

In mammals, the double-strand DNA breakage results in the activation of two signalling pathways, namely the ATM and ATR kinases, which ultimately lead to cell cycle arrest. In order to repair the damage caused to chromosomes, two distinct DNA repair pathways are initiated: either homology-directed repair (HDR) or nonhomologous end joining (NHEJ). The objective of these pathways is to maintain the integrity of the genome. The aforementioned DNA damage response pathways, namely DNA damage signalling and DNA repair, can become aberrantly activated at chromosome ends when telomeres are dysfunctional (Figure 9) (13). The issue of end protection can be addressed through the utilisation of shelterin and the formation of the T-loop structure. The two DNA damage signalling pathways are repressed independently by TRF2 and Pot1. This indicates that the ATM kinase pathway is inhibited by TRF2, whereas the ATR kinase pathway is prohibited by Pot1 (15, 22). In mammals, the two DNA repair pathways, non-homologous end joining (NHEJ) and homologous recombination (HR), can also be inhibited by TRF2 and Pot1. The fusion of two telomeres can result in

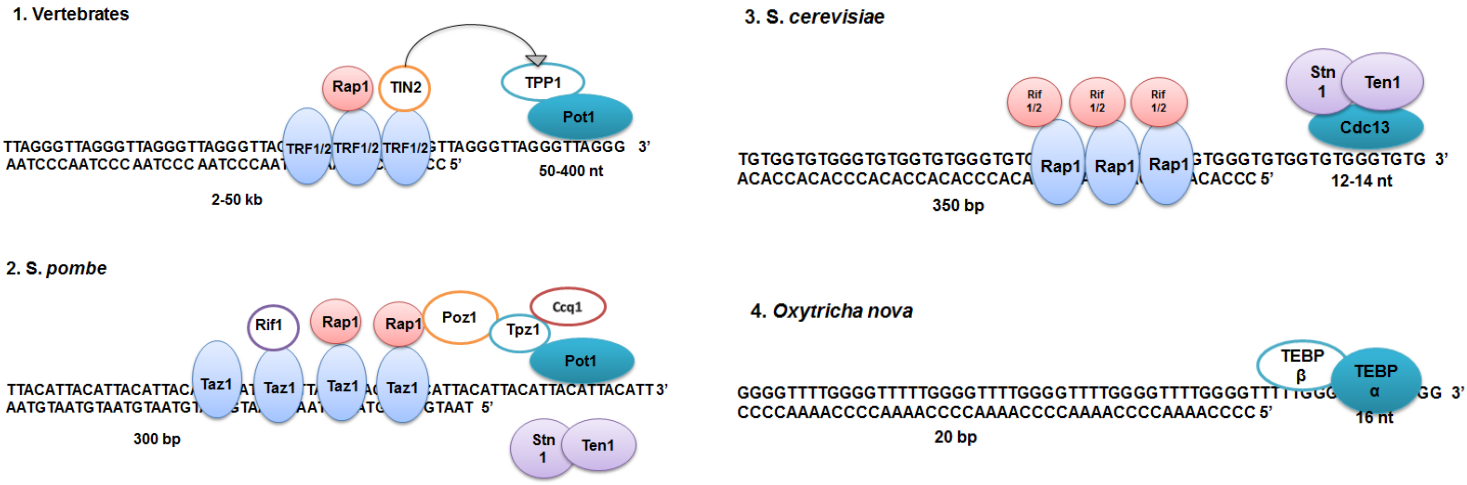

Figure 8. Comparison of shelterin to telomeric proteins in other eukaryotes. The proteins with the same colour could have similarity in their functions, whereas, similarity could not be observed in the case of their sequence and their structure. Mammals: Shelterin complex of TRF1, TRF2, Rap1, TIN2, TPP1, and POT1. Fission yeast: Shelterin-like complex of Taz1, Rap1, Poz1, Tpz1, Ccq1, and Pot1; also Ten1 and Stn1 are present. Budding yeast: dsDNA-binding complex of Rap1, Rif1, and Rif2; ssDNA- binding complex of Cdc13, Stn1, and Ten1. Oxytricha nova: TEBP α/β.

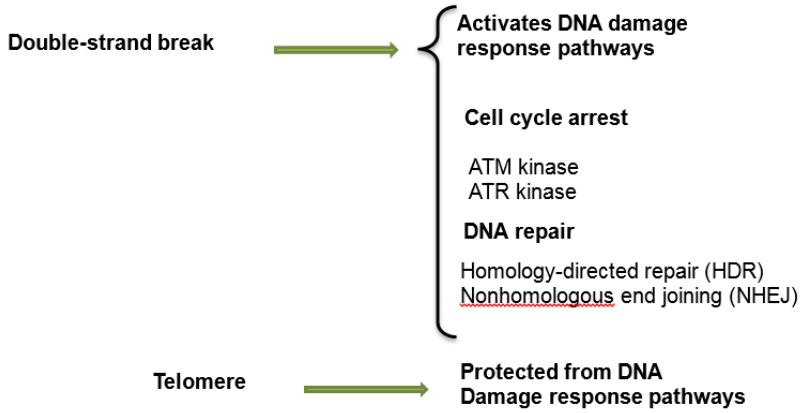

Figure 9. The end-protection problem. When a mammalian chromosome breaks, two signalling pathways can be activated (the ATM and ATR kinase pathways) which can induce cell death. One of two different DNA repair pathways (HDR and NHEJ) repairs the broken chromosome. Activation of DNA repair or DNA damage signalling has a problem for the ends of linear chromosomes, thus, telomeres can solve this end-protection problem by shelterin.

the formation of dicentric chromosomes through the action of the non-homologous end joining (NHEJ) pathway. Consequently, prior to DNA replication, during the G1 phase of the cell cycle, NHEJ is inhibited by TRF2 in the telomeres (24). However, following DNA replication, in the G2 phase of the cell cycle, NHEJ can be inhibited by TRF2 and Pot1. Furthermore, HDR can be impeded by TRF2, Pot1, and Ku70/80. Ku70/80 is a DNA repair factor and a component of the non-homologous end joining (NHEJ) pathway (44). In fission yeast, Taz1 has been demonstrated to inhibit the NHEJ pathway in a manner analogous to that observed for TRF2 in mammals. In budding yeast, Rap1 has been demonstrated to inhibit NHEJ, while Cdc13 has been shown to inhibit Mec1 kinase, which is equivalent to ATR kinase in humans. Otherwise, the cell cycle will arrest in the G2 to M transition (Figure 10) (13).

2.7. The T-Loop Structure

The folding of telomeres in several species has been observed to occur in a structure known as a t-loop, which is postulated to play a role in the protection of telomeres. In addition to vertebrates, t-loops have been identified in the ciliate Oxyricha fallax and the flagellate protozoan Trypanosoma brucei. They have also been observed in Pisum sativum (peas). However, there is currently no information available regarding the presence of t-loops in S. cerevisiae and S. pombe. The difficulty in isolating telomeric DNA in these two organisms is due to the fact

that they possess relatively short telomeres, measuring approximately 1 kb (11). In this structure, the duplex DNA portion of the telomere is invaded by the 3' telomeric overhang, which results in the chromosome end being hidden from the DNA damage checkpoint machinery. In this context, the recruitment of telomerase is also constrained; the telomeric protein TRF2 has been demonstrated to facilitate this formation (1). The ATM kinase signalling pathway, the non-homologous end joining (NHEJ) pathway and the ataxia telangiectasia and Rad3-related (ATR) signalling kinase pathway are all blocked by TRF2 and Pot1, which creates a t-loop structure.

Additionally, T-loops can impede the binding of telomere ends to Ku70/80 and MRN (a DNA end sensor). In the absence of TRF2, the T-loop is opened, resulting in the activation of both Ku70/80 and MRN. This leads to the activation of the NHEJ and ATM kinase pathways, respectively (Figure 11a) (13). In order to prevent the ATR kinase pathway, RPA (a single-stranded DNA-binding protein) must be inhibited in its binding to single-stranded DNA by Pot1. This indicates that the ATR pathway is activated by RPA in the absence of Pot1. Both the ATM and ATR kinase pathways induce cell cycle arrest at either the G1 to S or G2 to M transition, respectively (Figure 11b) (11).

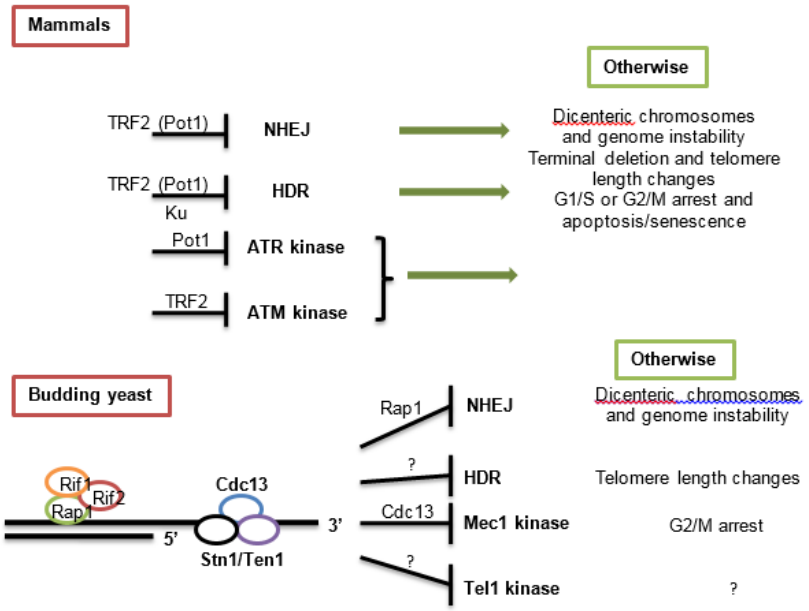

Figure10. Different solutions of the end protection problem. POT1 inhibits the ATR kinase signalling pathway and NHEJ especially after DNA replication, however, TRF2represses the ATM kinase signalling pathway and NHEJ at telomeres. Both POT1 and TRF2 inhibit HDR at telomeres. In budding yeast, Rap1 represses NHEJ and also Cdc13 inhibits Mec1 kinase.

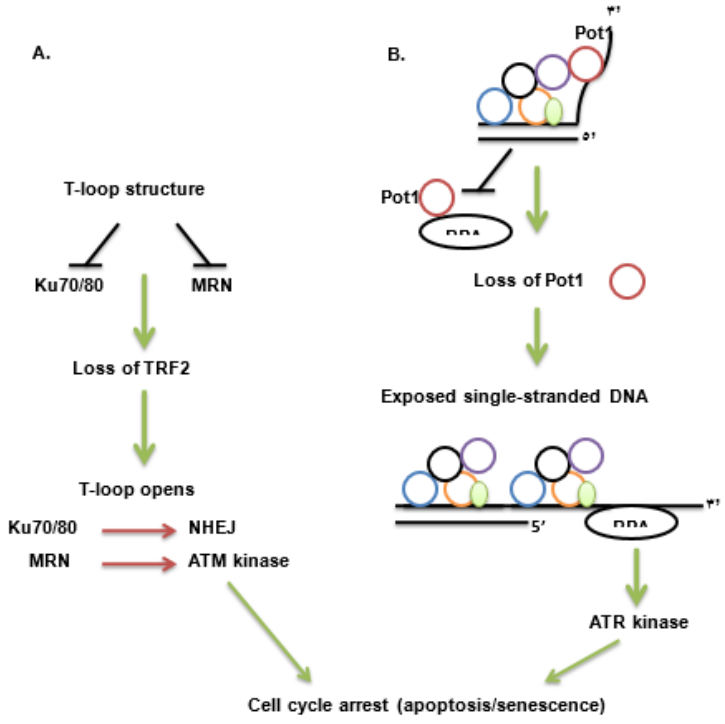

Figure 11. Different components of shelterin are dedicated to different aspects of the end-protection problem. (A), T-loop structure blocks telomeres end from the MRN (DNA end sensr) that activates the ATM kinase pathway, and the Ku70/80 that activates NHEJ. In (B), POT1 can repress ATR kinase signalling by inhibiting the binding of RPA that activates the ATR kinase pathway.

3. Conclusion

The present study is concerned with a comparative analysis of telomere structures across a range of eukaryotic organisms, including Saccharomyces cerevisiae, Saccharomyces pombe, and mammals. Telomeres are essential for preventing chromosome end fusion and consist of repetitive DNA sequences and associated proteins. In S. cerevisiae, the maintenance of telomeres is dependent on the activity of several proteins, including Rap1, Rif1, Rif2, Cdc13, Ten1, and Stn1. S. pombe employs a similar set of proteins, including Taz1, Rap1, Rif1, Pot1, Tpz1, Poz1, Ccq1, Stn1, and Ten1, to perform analogous functions. In mammals, the shelterin complex, comprising TRF1, TRF2, TPP1, POT1, TIN2, and Rap1, plays a pivotal role. Furthermore, the study examines the regulation of the shelterin complex and the activation of DNA damage response pathways in mammals, with a particular focus on double-strand breaks. Furthermore, the text considers the t-loop structure, a protective configuration of telomeres observed in a range of species. An understanding of these mechanisms reveals the evolutionary diversity and conservation of telomere maintenance systems, thereby providing insights into chromosome stability and cellular ageing across eukaryotes.

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Authors' Contribution

Study concept and design: M, S.
Acquisition of data: M, S.
Analysis and interpretation of data: M. S., M, M.
Drafting of the manuscript: M, S., M, M.
Critical revision of the manuscript for important intellectual content: M, M.
Administrative, technical, and material support: M, S.

Ethics

We hereby affirm that all ethical standards have been observed in the preparation of the submitted article.

Conflict of Interest

In the interest of transparency and to ensure the integrity of the research process, the corresponding author on behalf of all authors states that there is no conflict of interest.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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