

Review

To Fix or Not to Fix: Maintenance of Chromosome Ends Versus Repair of DNA Double-Strand Breaks

Erika Casari [†] , Marco Gnugnoli [†] , Carlo Rinaldi, Paolo Pizzul , Chiara Vittoria Colombo , Diego Bonetti and Maria Pia Longhese ^{*} 

Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano–Bicocca, 20126 Milano, Italy

^{*} Correspondence: mariapia.longhese@unimib.it; Tel.: +39-02-64483425

[†] These authors contributed equally to this work.

Abstract: Early work by Muller and McClintock discovered that the physical ends of linear chromosomes, named telomeres, possess an inherent ability to escape unwarranted fusions. Since then, extensive research has shown that this special feature relies on specialized proteins and structural properties that confer identity to the chromosome ends, thus allowing cells to distinguish them from intrachromosomal DNA double-strand breaks. Due to the inability of conventional DNA replication to fully replicate the chromosome ends and the downregulation of telomerase in most somatic human tissues, telomeres shorten as cells divide and lose this protective capacity. Telomere attrition causes the activation of the DNA damage checkpoint that leads to a cell-cycle arrest and the entering of cells into a nondividing state, called replicative senescence, that acts as a barrier against tumorigenesis. However, downregulation of the checkpoint overcomes this barrier and leads to further genomic instability that, if coupled with re-stabilization of telomeres, can drive tumorigenesis. This review focuses on the key experiments that have been performed in the model organism *Saccharomyces cerevisiae* to uncover the mechanisms that protect the chromosome ends from eliciting a DNA damage response, the conservation of these pathways in mammals, as well as the consequences of their loss in human cancer.

Keywords: telomere; double-strand breaks; checkpoint; senescence; *S. cerevisiae*; cancer



Citation: Casari, E.; Gnugnoli, M.; Rinaldi, C.; Pizzul, P.; Colombo, C.V.; Bonetti, D.; Longhese, M.P. To Fix or Not to Fix: Maintenance of Chromosome Ends Versus Repair of DNA Double-Strand Breaks. *Cells* **2022**, *11*, 3224. <https://doi.org/10.3390/cells11203224>

Academic Editor: Antonio Porro

Received: 15 September 2022

Accepted: 12 October 2022

Published: 14 October 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. History of the Discovery of Telomeres and Telomerase

The concept of telomere was born in the first half of the twentieth century, when Hermann J. Muller, working with the fruit fly *Drosophila melanogaster*, found that X-rays could generate broken chromosomes that can fuse to each other leading to inversions, deletions, and/or translocations (Figure 1). However, these structural alterations never involved the chromosome termini. This finding led him to propose the existence of a “terminal gene” that “must have a special function, that of sealing the ends of the chromosome, so to speak, and that for some reason, a chromosome cannot persist indefinitely without having its ends thus sealed” [1]. Muller called this gene “telomere”, from the Greek *telos* “end” and *meros* “part”.

The idea that the telomere conferred identity to the natural ends of a chromosome, such that a cell could distinguish them from the ends of intrachromosomal double-strand breaks (DSBs), was confirmed soon thereafter by Barbara McClintock, during her cytological studies on irradiated maize chromosomes. She found that a broken chromosome frequently fused with another broken end to produce a dicentric chromosome that would break at the next mitosis, when the two centromeres are pulled apart toward opposite poles of the mitotic spindle. Such broken ends can fuse with other broken ends, starting the so-called “breakage-fusion-bridge cycle” [2]. However, she did not detect fusions involving telomeres, suggesting that one function of the telomere was to protect the natural chromosome ends from fusing to each other.

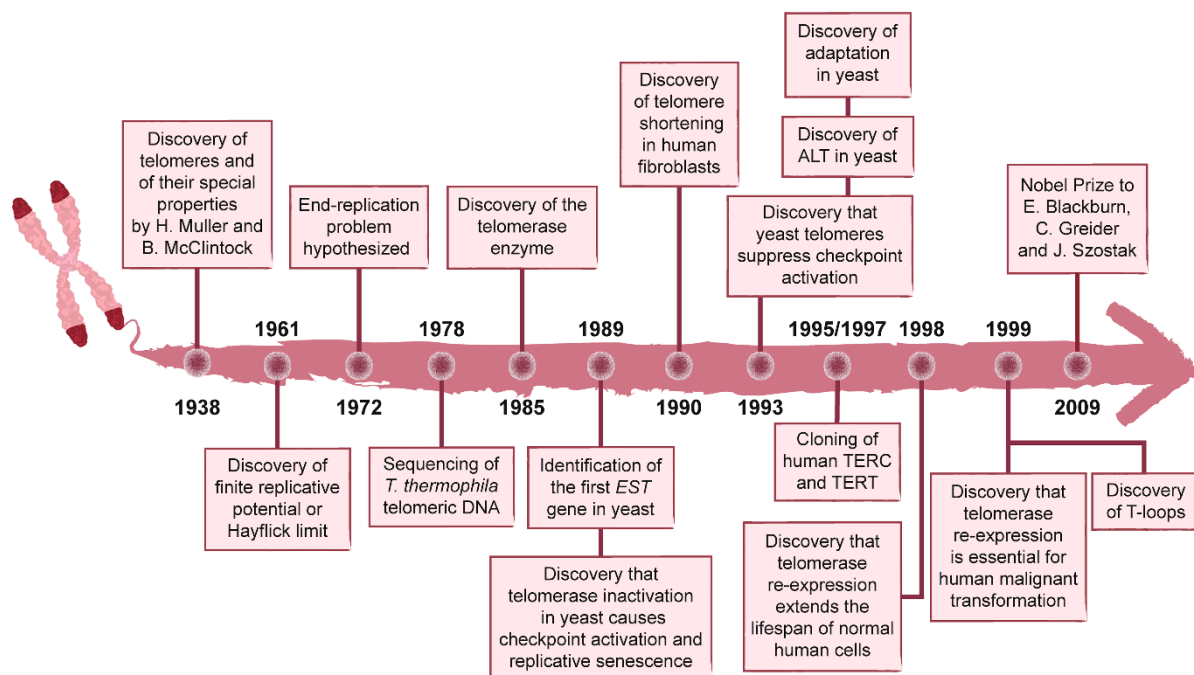


Figure 1. Timeline of the major discoveries in the telomere field.

Following the discovery that telomeres should have special properties, a critical experiment to study telomere function was performed in *Saccharomyces cerevisiae* by Sandell and Zakian, who placed the recognition site of the HO endonuclease 20 kb away from the dispensable left telomere of chromosome VII, such that the telomere can be lost upon HO expression [3]. After elimination of the telomeric DNA, they found that cells underwent an arrest of cell-cycle progression due to activation of the DNA damage checkpoint, indicating that telomeres were essential to prevent the natural chromosome ends from being recognized as DSBs by the checkpoint machinery.

The sequence of telomeric DNA was first identified by Blackburn and Gall in the ciliated protozoan *Tetrahymena thermophila*. They found that the ends of macronuclear ribosomal DNA (rDNA) molecules in this organism consisted of a variable number of 5'-CCCAA-3' repeats [4]. Sequencing of the telomeric DNA from other eukaryotic organisms revealed that as in *T. thermophila*, the ends of chromosomes were constituted by a block of tandemly repeated simple sequences, whose number varied depending on the organism. Furthermore, the composition of telomeric DNA was asymmetric, with the DNA strand running in the 5' to 3' direction rich of guanine and longer than the complementary strand [5].

The fact that telomeres from the same organism could be of different lengths suggested that telomeric DNA was not templated by the parental chromosome. This hypothesis was strengthened by Szostak and Blackburn, who ligated *T. thermophila* rDNA telomeres onto both ends of a linear yeast plasmid and introduced it into yeast by transformation [6]. They found that yeast cells maintained the plasmid as a linear molecule. Furthermore, cells were capable to add the yeast C₁₋₃A telomeric repeats at the tips of *T. thermophila* rDNA telomeres [7], arguing that the structural features required for telomere replication had been conserved during evolution.

The finding that telomere elongation was entirely attributable to an increase in the number of tandemly repeated units led Blackburn and Szostak to propose the existence of a terminal transferase-like activity that was capable to add telomeric repeats onto chromosome ends, and that *T. thermophila* DNA ends, but not random sequence, could be recognized as a substrate by the “DNA addition enzyme” [7]. By using cell-free extracts from *T. thermophila*, Greider and Blackburn first uncovered the existence of a terminal transferase activity capable of adding DNA repeat sequences to the chromosome ends [8].

Purification of this enzyme, named telomerase, allowed them to show that it was a ribonucleoprotein complex, whose RNA and protein components were both essential for its activity [9]. The RNA component was then cloned and it was found to contain a short RNA sequence that could act as a template for the addition of simple repeats [10]. Mutation of this sequence caused the introduction, in vivo, of telomere sequences corresponding to the mutated sequence, thus providing the proof that telomerase used its integral RNA component as the template for the addition of simple repeat units [11].

In parallel, the search for *S. cerevisiae* mutants that were impaired in the ability to convert a circular plasmid containing inverted repeats of *T. thermophila* telomeric sequences into a stable linear form allowed Lundblad and Szostak to discover the first gene encoding one of the subunits of the yeast telomerase enzyme [12]. This gene was called *EST1*, because its mutation led to loss of telomeric DNA, giving rise to the so-called “ever shorter telomere” (est) phenotype. *est1* mutants also showed a gradual decline of cell viability, thus providing the first experimental demonstration that loss of telomeric DNA limits cellular proliferation. As the lack of Est1 caused chromosome loss, three additional *EST* genes (*EST2*, *EST3*, and *EST4*) were discovered in a screen for mutants that exhibited increased chromosome instability combined with defects in plasmid linearization [13]. The *TLC1* (telomerase component 1) gene encoding the template RNA of telomerase was identified by Singer and Gottschling in a search for genes that, when overexpressed, counteract the ability of telomeres to silence transcription [14]. Purification of the catalytic subunit of telomerase from a ciliated protozoan allowed to demonstrate that the reverse transcriptase motifs were essential for telomeric DNA synthesis in vivo and in vitro [15]. This discovery was followed by the cloning of human telomerase RNA component (TERC) [16] and the telomerase reverse transcriptase (TERT) [17].

2. The DNA Damage Response

Although telomeric DNA is structurally similar to the end of a DSB, it is intrinsically refractory to repair and does not activate the DNA damage response (DDR) that is, instead, elicited by an intrachromosomal DSB. In eukaryotic cells, the DDR comprises pathways to repair DNA breaks and a mechanism, called DNA damage checkpoint, that inhibits cell-cycle progression until DNA lesions are repaired [18]. The main mechanisms repairing a DSB are non-homologous end-joining (NHEJ) and homologous recombination (HR) (Figure 2). NHEJ catalyzes the direct ligation of the DSB ends and requires the Ku complex that comprises the two Ku70 and Ku80 subunits. This protein complex acts as a hub for the recruitment of downstream NHEJ components, including *S. cerevisiae* Lif1 (human XRCC4), Nej1 (human XLF), and the DNA ligase IV [19]. The presence of Ku at DSBs also protects the DNA ends from degradation by inhibiting the recruitment of the Exo1 nuclease [20,21]. By contrast, HR is a more complex process that uses undamaged homologous DNA as a template to restore the genetic information lost at the break site [22].

The key step in determining which pathway is used to repair a DSB is the initial nucleolytic degradation of the DSB ends. While NHEJ requires little or no DSB end-processing, initiation of HR requires that the 5'-terminated strands of the DSB ends are nucleolytically degraded, in a process called resection, to generate 3'-ended single stranded DNA (ssDNA) [23]. Extended resection of the DSB ends not only commits DSB repair to HR, but it makes also the DNA ends refractory to be ligated by the NHEJ machinery. The resulting 3'-ended ssDNA is first coated by the ssDNA binding complex replication protein A (RPA), which is then replaced by the recombinase Rad51 to form a right-handed helical nucleoprotein filament for homology search and strand invasion (Figure 2). The invading DNA end serves to prime DNA synthesis using the intact homologous DNA sequence as a template, followed by resolution of the resulting DNA structure and ligation [22].

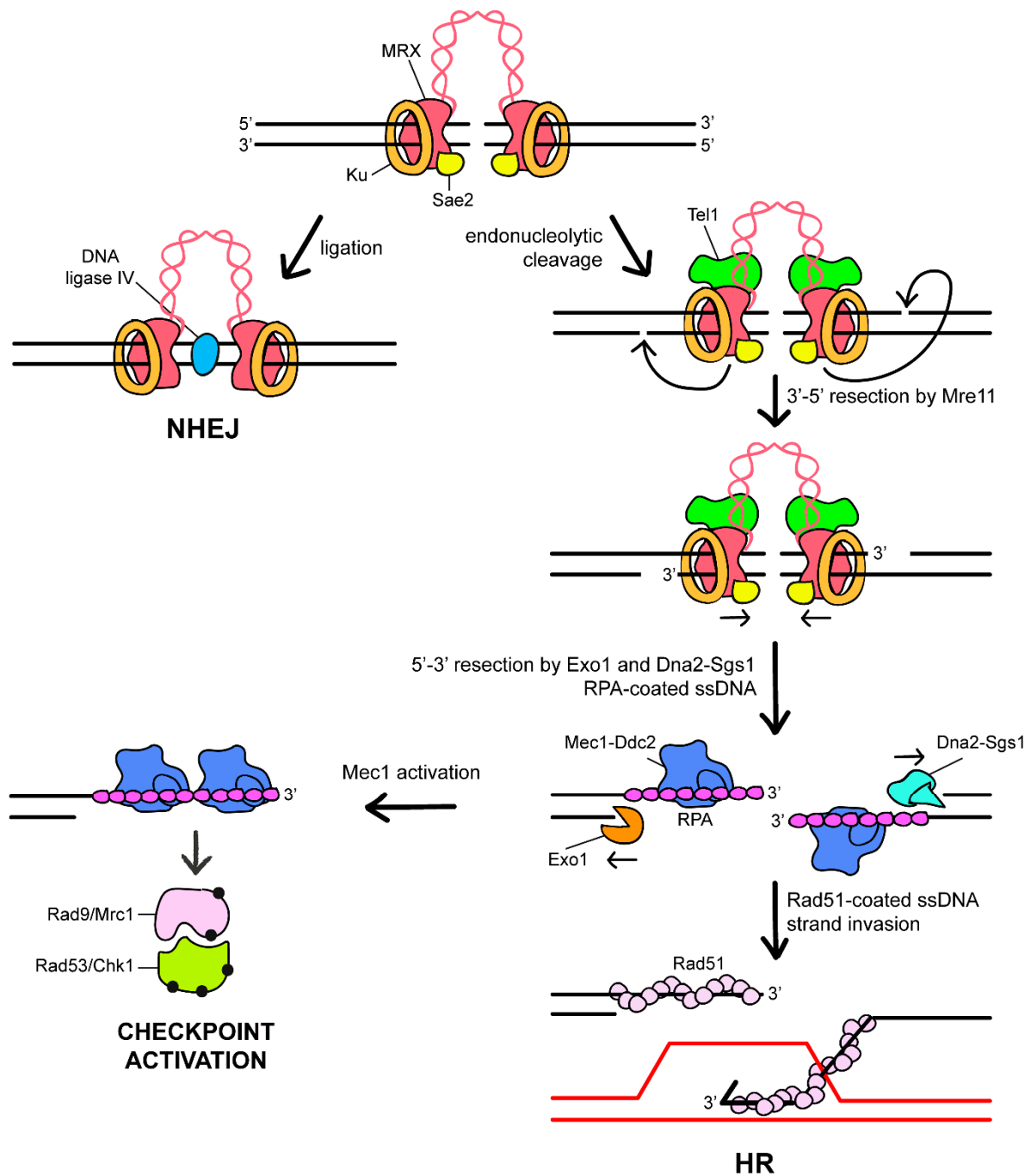


Figure 2. Overview of the DDR at DSBs in *S. cerevisiae*. DSBs can be repaired by non-homologous end-joining (NHEJ) or homologous recombination (HR). MRX-Sae2 and Ku protein complexes are recruited to the DSB. MRX is required to load Tel1. In NHEJ, Ku acts as a hub to recruit downstream NHEJ components, including the DNA ligase IV that catalyzes direct ligation of the DSB ends. If the DSB is not repaired by NHEJ, upon ATP hydrolysis by Rad50, Mre11 together with Sae2 catalyzes an endonucleolytic cleavage of the 5'-terminated strands, followed by bidirectional resection catalyzed by Mre11 in the 3' to 5' direction and by Exo1 or Dna2-Sgs1 in the 5' to 3' direction. RPA binds to the 3'-ended ssDNA overhangs and is then replaced by Rad51. The Rad51-ssDNA intermediate initiates the homology search, invades the dsDNA, and pairs with the homologous DNA strand. RPA-coated ssDNA recruits the Mec1-Ddc2 complex, which eventually leads to checkpoint activation by activating Rad53 and/or Chk1 through the adaptor Rad9 or Mrc1. Black dots indicate phosphorylation events.

In both yeast and mammals, DSB resection requires the evolutionarily conserved MRX/MRN protein complex that is composed of Mre11, Rad50, and Xrs2 (human MRE11, RAD50, and NBS1) subunits [24] (Table 1). This complex possesses a hetero-hexameric structure, in which Mre11 dimerizes and interacts with both Rad50 and Xrs2/NBS1. While Mre11 exhibits 3'-5' exonuclease and endonuclease activities [25,26], Rad50 is an ATPase that possesses two antiparallel coiledcoil domains that can dimerize through a Zn-hook motif [24]. During DSB resection, the Sae2 (human CtIP) protein stimulates a latent Mre11 endonuclease activity within the context of the MRX complex to cleave the 5'-terminated strands on either side of the DSB [27]. The resulting nick generates an entry site for Mre11 exonuclease, which degrades back toward the DSB end in a 3'-5' direction, and for Exo1 and Dna2 nucleases that degrade DNA away from the DSB in a 5'-3' direction [28–35] (Figure 2). Dna2 processing activity requires the RecQ helicase Sgs1 (human BLM) that unwinds double-stranded DNA (dsDNA) and generates a substrate for Dna2 that cleaves ssDNA overhangs adjoining duplex DNA (Table 1). The ATPase activity of Rad50 drives conformational changes of the complex that modulate its functions. In particular, the Rad50 dimer, when bound to ATP, prevents the access of Mre11 to dsDNA [36–39], whereas ATP hydrolysis induces a dissociation of the Rad50 nucleotide binding domains and the reposition of Mre11 to one side of Rad50 dimer. This conformational change generates a DNA cutting channel that allows Mre11 to bind dsDNA and to endonucleolytically process it [37,40].

Table 1. Major proteins involved in the DNA damage response and telomere capping.

<i>S. cerevisiae</i>	<i>H. sapiens</i>	Description
Mre11-Rad50-Xrs2	MRE11-RAD50-NBS1	DSB sensor; telomere length regulator
Ku70-Ku80	KU70-KU80	DSB sensor; telomere length regulator
Tel1	ATM	Apical protein kinase; telomere length regulator
Sae2	CtIP	Activator of MRX/MRN endonuclease
Exo1	EXO1	Exonuclease
Sgs1	BLM	DNA helicase
Dna2	DNA2	DNA helicase and nuclease
Mec1-Ddc2	ATR-ATRIP	Apical protein kinase and interacting factor
Rad9	53BP1	Checkpoint adaptor/mediator
Mrc1	Claspin	Replisome component; checkpoint activator
Rad53	CHK2	Downstream protein kinase
Chk1	CHK1	Downstream protein kinase
Cdc13-Stn1-Ten1	CTC1-STN1-TEN1	Telomere binding complex; telomere capping regulator
Rap1-Rif1-Rif2	TRF1-TRF2-RAP1-TIN2-TPP1-POT1	Telomere binding complex; telomere capping and length regulator

DSB occurrence can elicit activation of a DNA damage checkpoint response, which couples DSB repair with cell-cycle progression [18]. Key checkpoint players include the apical protein kinases Tel1 and Mec1, whose mammalian orthologs are ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3-related), respectively (Table 1). Tel1, which was originally identified for its requirement to elongate *S. cerevisiae* telomeres [41], is the kinase involved in sensing and signaling unprocessed or minimally processed DNA DSBs. In both yeast and mammals, recruitment and activation of Tel1/ATM require the MRX/MRN complex [42–46]. Tel1, in turn, once loaded at DSBs by MRX, supports MRX function in a positive feedback loop by promoting/stabilizing its association to the DSB [47].

Upon DSB resection, the replication protein A (RPA) complex binds the ssDNA overhangs and promotes recruitment of Mec1/ATR kinase [48]. Mec1, as well its human ortholog ATR, interacts with Ddc2 (human ATRIP) that helps its recruitment to the DSB ends [49]. Once Mec1/ATR is activated by RPA-coated ssDNA, it phosphorylates and activates the downstream checkpoint kinases Rad53 (human CHK2) and Chk1 (human CHK1), which control two parallel branches of the checkpoint [50]. Signal transduction from apical to downstream checkpoint kinases requires the mediator proteins Rad9 (human 53BP1) and Mrc1 (human Claspin) (Figure 2) (Table 1). In particular, Rad9 allows Rad53

phosphorylation and checkpoint activation in response to DNA damage in the G1 and G2 phases [51,52], whereas Mrc1, which is a component of the replisome, promotes Rad53 activation during S phase [53–56].

3. Telomere Capping and the Consequence of Its Loss

Following the discovery that the ends of chromosomes should possess a unique structure that prevents their fusion, *S. cerevisiae* cells have been used to demonstrate that, when a short array of telomeric DNA repeats was inserted immediately adjacent to an endonuclease-induced DSB, the break was not subjected to fusions by NHEJ because of its failure to recruit the DNA ligase IV [57–59]. Furthermore, this DSB was unable to elicit a checkpoint response [57,58], indicating that telomeres exert an “antichkpoint” activity. Subsequent studies have established that suppression of DNA repair and DNA damage checkpoint at telomeres, referred to as capping, relies on proteins specifically present or enriched at single-stranded and double-stranded telomeric DNA that, in budding yeast, include the protein complexes Cdc13-Stn1-Ten1 (CST), Ku70-Ku80 (Ku), and Rap1-Rif1-Rif2 (Table 1).

3.1. The CST Complex

The earliest demonstration of the existence of specialized proteins that distinguished the chromosome ends from internal DSBs was the discovery that yeast cells, carrying a temperature-sensitive mutation in the *CDC13* gene, incubated at restrictive temperatures, degraded their telomeres resulting in extensive ssDNA that activates a Rad9/Mec1-dependent checkpoint [60,61]. Cdc13 interacts with Stn1 and Ten1 proteins to form a telomeric ssDNA binding complex called CST (Figure 3A). Both Stn1 and Ten1 support Cdc13 capping activity. In fact, exposure of cells harboring *stn1* or *ten1* conditional alleles to restrictive conditions causes telomere degradation and checkpoint-mediated cell-cycle arrest [62–64]. This protein complex is highly conserved and has been identified in ciliates, vertebrates, flies, and plants [65].

Subsequent studies have shown that the CST complex has structural similarities with the single-strand DNA binding complex RPA [66], but with a preferential binding to telomeric G-rich ssDNA overhangs. As Mec1 recognizes RPA-coated ssDNA, CST has been proposed to inhibit Mec1 activation by blocking RPA from gaining access to the telomeric single-stranded overhangs, thus limiting Mec1 loading onto DNA and, therefore, its activation [67].

The use of an inducible degron allele of Cdc13 allowed to demonstrate that the CST complex exerts its capping function during late S and G2/M phases of the cell cycle, but not in G1 or early S [68,69]. Interestingly, passage through S phase in a temperature sensitive *cdc13* mutant causes generation of Exo1-dependent ssDNA and unstable chromosomes that are then the source for additional chromosome instability events [70]. This genome instability has been shown to be due to defects in telomere replication, suggesting that the Cdc13 capping function relies on its involvement in supporting replication of telomeric DNA. Consistent with this hypothesis, Cdc13 and Stn1 physically interact with the pol α -primase complex and promote its recruitment to the telomeric DNA to fill-in the C-strand [71–73]. As telomere binding proteins can represent intrinsic obstacles for replication fork progression [74,75], the role of CST in supporting pol α -primase activity could facilitate repriming on the lagging strand to compensate for fork stalling that inherently occurs during telomere replication.

The CST complex in mammals is comprised of CTC1, STN1, and TEN1 subunits [65] (Figure 3B). When CTC1 is disrupted, the G-rich 3'-ended overhangs elongate, while the C-strands decrease in length due to a deficiency in C-strand fill-in synthesis [76–78]. The role of CST as a repriming complex at telomeres was also proposed for the mammalian CST [79,80], which appears to have extratelomeric functions in DNA replication and fork restart in the presence of replication stress [81].

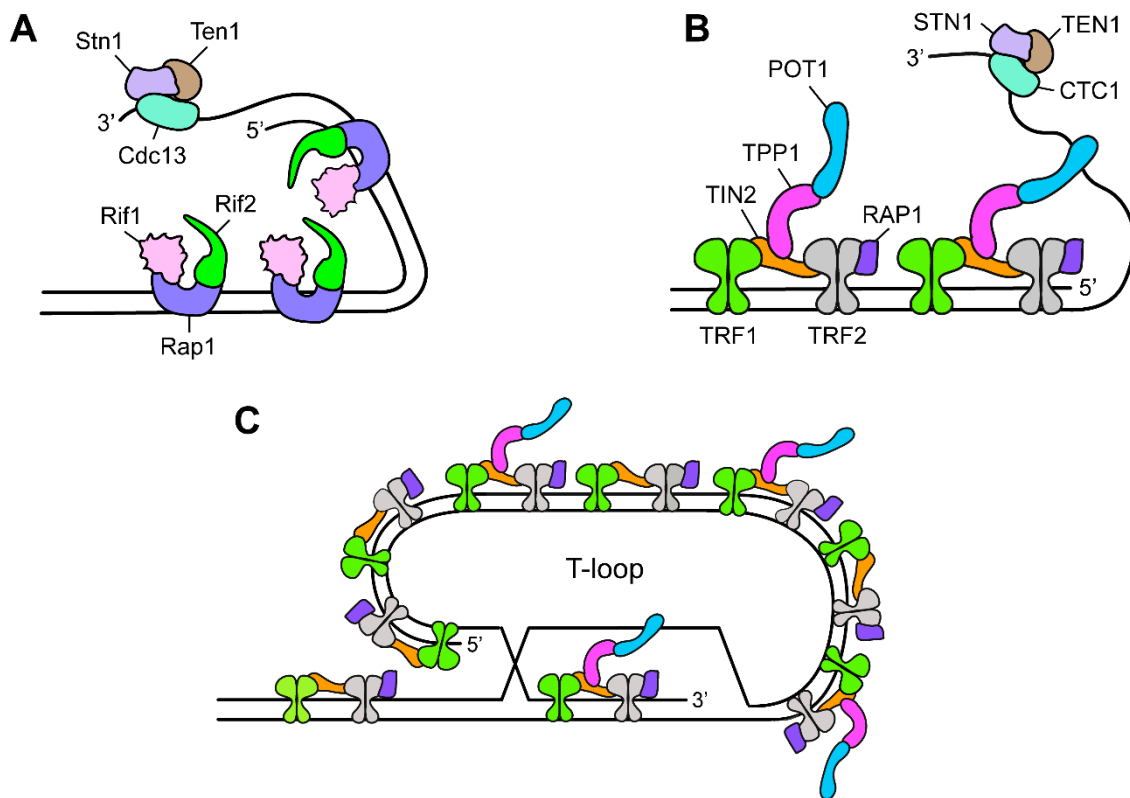


Figure 3. Telomeric structure and capping proteins in yeast and humans. **(A)** Schematic representation of the yeast Rap1-Rif1-Rif2 and CST complexes. CST in yeast is composed of Cdc13, Stn1, and Ten1 proteins. **(B)** Schematic representation of the mammalian shelterin complex, composed of TRF1, TRF2, TIN2, RAP1, TPP1, and POT1 subunits, and the CST complex, composed of CTC1, STN1 and TEN1 subunits. **(C)** The G-rich 3'-ended single stranded overhang bound by the shelterin complex is looped back into the telomeric DNA to form a telomeric loop (T-loop).

3.2. The Ku Complex

In the G1 phase of the cell cycle, telomere capping relies on the Ku complex, which is an evolutionarily conserved heterodimer composed of Ku70 and Ku80 (human KU70 and KU80) subunits. In *S. cerevisiae*, Ku restrains degradation of telomeric DNA and checkpoint activation [82–84]. The increased ssDNA and the activated checkpoint response in cells lacking any Ku subunit at elevated temperatures can be suppressed by deletion of *EXO1* [83], suggesting that Ku represses Exo1 activity at telomeres. Consistent with this hypothesis, the phenotypes caused by Ku dysfunction can be suppressed also by the overexpression of either Est2 reverse transcriptase or TLC1 RNA template, whose high levels appear to stabilize telomeres by enhancing their resistance to degradation by Exo1 [85,86]. The Ku complex is constitutively present also at human telomeres, where it protects telomeric DNA from degradation and HR, although this repression in Ku-deficient mouse cells involves also RAP1 and POT1 proteins that belong to the shelterin complex (see next paragraph) [87–89].

3.3. The Rap1-Rif1-Rif2 Complex

In *S. cerevisiae*, the other protein complex with capping function is composed of Rap1, Rif1, and Rif2 proteins, with Rap1 binding directly double-stranded telomeric DNA (Figure 3A). These proteins also negatively regulate telomere length by controlling different pathways [90,91]. In *S. cerevisiae*, Rap1 and Rif2, and to a much lesser extent Rif1, repress telomere-telomere fusions by NHEJ, telomere degradation, and checkpoint activation [58,68,84,92]. Rif2 also inhibits activation of Tel1, which is known to promote telomerase-mediated telomere elongation [93,94]. By contrast, Rif1, but not Rif2, is impor-

tant to support viability in cells where Cdc13 is dysfunctional [95]. Thus, Rap1 and its interactors Rif1 and Rif2 have capping activities, with Rif1 and Rif2 making specific and separable contributions to this capping.

In mammals, the capping properties of the Rap1-Rif1-Rif2 complex are functionally recapitulated by a protein complex, called shelterin, which is composed of TRF1, TRF2, RAP1, TIN2, TPP1, and POT1 subunits [96] (Figure 3B). TRF1 and TRF2 bind to TIN2. TIN2 also binds to TPP1, which in turn binds to POT1, whereas RAP1 binds TRF2. RAP1 is the only shelterin subunit possessing a limited conservation with its yeast ortholog Rap1, although yeast Rap1 binds directly telomeric DNA, whereas the association of human RAP1 with DNA is mediated by TRF2.

The shelterin complex uses a variety of strategies and different subunits to block the DDR activities at telomeres. In particular, TRF2 prevents ATM activation, whereas POT1 is used to repress ATR signaling activity [97]. As POT1 binds ssDNA, the proposed model is that POT1 blocks RPA from gaining access to the telomeric single-stranded overhang, thereby limiting ATR activation. TRF2 is also the main inhibitor of classical NHEJ (c-NHEJ), a pathway responsible for the generation of end-to-end fusions and dicentric chromosomes that can result in breakage-fusion-bridge cycles and genome instability [98]. Artificial tethering of TRF2 next to a DSB impedes its repair and elicits prolonged DDR activation, suggesting that TRF2 is both necessary and sufficient to suppress c-NHEJ [59]. The main mechanism through which TRF2 exerts this inhibitory function is based on formation of T-loops, which are large lariat structures that are generated through strand invasion of the long 3'-ended overhang into the double-stranded telomeric DNA [99,100] (Figure 3C). TRF2 was also found to limit ATM signaling directly by inhibiting the kinase activity itself [101].

The shelterin complex also represses an end-joining pathway, called alternative NHEJ (alt-NHEJ), which is mediated by poly(ADP-ribose) polymerase 1 (PARP1), DNA ligase III, and the error-prone translesion DNA polymerase θ [102]. While c-NHEJ leads to minimal sequence alterations, alt-NHEJ causes extensive deletions and insertions at the repair junction. Furthermore, it is responsible for telomere fusions in senescent cultured cells and in human cancers, suggesting that it might be involved in the processing of dysfunctional telomeres in the early stages of tumorigenesis [103]. Activation of alt-NHEJ can be observed only when all the shelterin subunits are completely depleted in mouse cells that lack the Ku complex [104], suggesting that repression of alt-NHEJ at telomeres involves multiple proteins that act in a redundant manner.

Although there are significant differences in the sequences and proteins at yeast and human telomeres, human TRF1 and TRF2 share with *S. cerevisiae* Rap1 the DNA binding domain with two Myb-like folds. However, while TRF1 and TRF2 contain a single Myb-like domain, *S. cerevisiae* Rap1 binds a recognition sequence through two tandem Myb-like domains [105–107]. In yeast Rap1, a wrapping loop, immediately after the C-terminal Myb-like domain, folds back and locks Rap1 around DNA by interacting with the Myb domain located at the N-terminus. The transient opening of the wrapping loop destabilizes this clamped structure and allows Rap1 binding to DNA through a single Myb-like domain [108,109]. These different Rap1 DNA binding modes were shown to influence Rap1 ability to interact with Rif2 in vivo [110]. In fact, Rap1 mutant variants that increase or decrease Myb affinity to DNA, as well as mutational impairments of the wrapping loop clamping, showed that binding of both Myb-like domains to DNA results in Rap1-DNA complexes that act primarily through Rif2 to control MRX functions at telomeres. By contrast, the transition to a binding mode where a single Myb-like domain is bound to DNA leads to Rap1-DNA complexes that inhibit MRX function at telomeres in a Rif2-independent manner [110].

In any case, the ability of Rif2 to counteract Tel1 activation, NHEJ, and nucleolytic degradation of telomeric DNA appears to rely on inhibition of MRX activity at telomeres [58,68,84]. Because Rif2 interacts with Xrs2 C-terminus within the same region as Tel1 [94], Rif2 was initially proposed to inhibit MRX association/persistence to telomeric DNA ends by competing with Tel1 for Xrs2 binding, therefore antagonizing Tel1-mediated stabilization of

MRX association with DNA ends. However, the finding that Rif2 interacts *in vitro* with Rad50 and can inhibit MRX-dependent stimulation of Tel1 kinase activity independently of Xrs2 [47,111], suggests that Rif2 can act directly on Rad50 to control MRX activity at telomeres. Interestingly, Rif2 was shown to stimulate ATP hydrolysis by Rad50 in an Xrs2-independent manner [47,111]. As MRX binding to DNA, as well as its ability to stimulate Tel1 activation and NHEJ requires that Rad50 is bound to ATP [36,39,112–114], Rif2 can inhibit all these MRX functions by discharging the MRX ATP-bound conformation through stimulation of Rad50 ATPase.

Sae2 is required to stimulate Mre11 endonuclease activity [27]. Interestingly, Rif2 was recently shown to inhibit Mre11 endonuclease activity within the context of the MRX complex [115,116]. Genetic and structural modelling approaches identified K6, N18, K81, and I93 residues on Rad50 as being important to support Rad50-Rif2 interaction and Rif2-mediated inhibition of Mre11 endonuclease [115–117]. Notably, two of these amino acids belong to a cluster of residues found to be mutated in the meiosis-defective *rad50-S* alleles, which specifically impair Mre11 endonuclease activity by abrogating Rad50-Sae2 interaction and, therefore, Sae2-mediated stimulation of Mre11 nuclease [118]. Altogether these findings suggest that Sae2 and Rif2 interaction interfaces can partially overlap on Rad50, raising the possibility that Rif2 can inhibit Mre11 endonuclease by competing with Sae2 for Rad50 binding and, therefore, by limiting Sae2-mediated MRX stimulation.

Although Rif2 can be detectable also at DSBs [47], it is much more abundant at telomeres compared to Sae2, which in turn is avidly bound to DSBs. The different enrichment of these two proteins at DSBs versus telomeres provides the rationale to explain why Rif2 inhibits MRX-mediated resection preferentially at telomeres. In fact, at DSBs Rif2 only represses Tel1 activation and NHEJ by discharging the MRX ATP-bound state [47], whereas it fails to inhibit resection possibly because it is not enough to antagonize Sae2 binding to Rad50 and, therefore, the conversion of MRX into an endonuclease active complex (Figure 4).

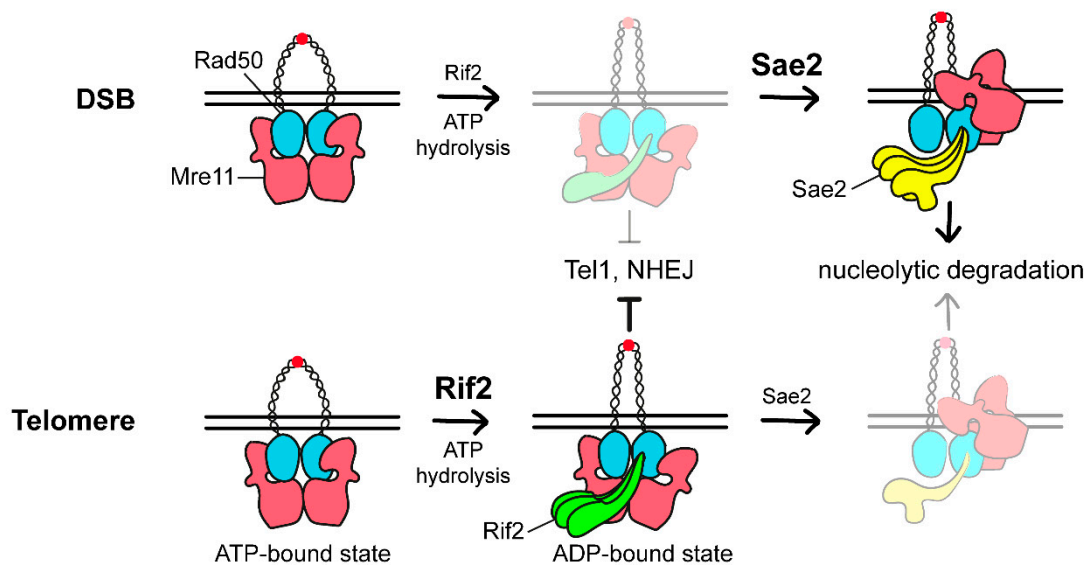


Figure 4. Model of regulation of MRX activity at DSBs and telomeres. In the ATP-bound state, the dsDNA is inaccessible to Mre11. Upon ATP hydrolysis by Rad50, the two Rad50 coiled coils zip up and Mre11 moves to the side of Rad50 dimer where it can act as endonuclease. At the DSB, the excess of Sae2 compared to Rif2 leads to Sae2 binding to the Rad50-Mre11 interface. This interaction stabilizes Mre11-Rad50 in a conformation that is proficient to cleave DNA. At telomeres, the excess of Rif2 compared to Sae2 antagonizes Sae2 binding to Rad50 and stabilizes a post hydrolysis ADP-bound state that is not competent for NHEJ, Tel1 activation, and DNA cleavage. Xrs2 is not represented.

Rif2 functions in inhibiting Mre11 endonuclease, Tel1 activation, and NHEJ depend on a small Rif2 region of as little as 34–40 amino acids, called MIN (MRN-INhibitor) [111,115,117].

The MIN motif, which belongs to the Rif2 BAT (Blocks Addition of Telomeres) motif (residues 1–60), previously shown to be involved in the negative regulation of telomere length [119], mediates also Rif2 binding to Rad50 and stimulation of ATPase activity. The finding that Rad50 K6 and K81 residues are evolutionarily conserved (human K6 and R83) raises the possibility that an analogous mechanism might be deployed in other eukaryotes.

4. Consequences of Telomere Shortening

Telomere attrition can be caused not only by disrupting the interaction between the telomeric DNA and the proteins that specifically bind to it, but also by eliminating the telomerase activity. In 1961, Leonard Hayflick discovered that human fibroblasts derived from fetuses possessed finite replicative potential of 50–60 doublings. Then, they entered a non-dividing state called replicative senescence or the “Hayflick limit” [120]. Based on the finding that DNA polymerases replicate DNA only in the 5′ to 3′ direction and need a primer to initiate DNA synthesis [121], Alexei Olovnikov, in its theory of marginotomy, predicted that the conventional DNA replication machinery cannot replicate completely the chromosome ends that would incur a loss of DNA from the lagging strand, leading to progressive chromosome shortening. He proposed that this shortening can account for the limitation of doubling potential of normal somatic cells [122].

S. cerevisiae cells possess a constitutively active telomerase, but a senescence phenotype can be induced following telomerase removal. The first experimental demonstration that loss of telomeric DNA limits cellular proliferation comes from the discovery that yeast cells lacking the Est1 subunit of telomerase showed a gradual arrest of doubling [12]. Similarly, human cultured primary cells shorten their telomeres as a function of serial passage during ageing [123]. Furthermore, re-expression of the catalytic subunit of telomerase can extend the lifespan of telomerase-negative human cells [124].

One hypothesis to explain the decrease in growth capacity was that chromosome erosion leads to genetic instability that causes cell death. Indeed, it was shown that yeast cells deleted for the *EST1* gene increase the frequency of gross chromosomal rearrangements involving terminal, but not internal, deletions [125]. However, these events can be detectable only after a significant loss of growth potential, suggesting that chromosome instability was not the major determinant of the senescent phenotype. Rather, loss of growth in these yeast cells correlated with an arrest of the cell cycle in the G2 phase and activation of Rad53 checkpoint kinase [126,127]. This response was dependent on genes involved in the DNA damage checkpoint, indicating that checkpoint activation at critically short telomeres is the trigger of replicative cellular senescence. Similarly, human cells, in which the telomerase activity is downregulated, undergo telomere shortening and prolonged DDR signaling, which results in the formation of telomere-induced DNA damage foci (TIFs) that colocalize with DNA repair and DNA damage checkpoint proteins [128,129]. Furthermore, overexpression of the shelterin subunit TRF2 can delay senescence onset [130], arguing that checkpoint activation at telomeres can be elicited not only as a consequence of telomerase inactivation but also of insufficient amounts of shelterin bound at telomeres.

Altogether, these findings lead to a model whereby as telomeres shorten, they become progressively unable to bind telomere-capping proteins, thus resembling one-ended DSBs (Figure 5). This change in the protected status leads to activation of a checkpoint that is similar to that triggered by intrachromosomal DSBs and that permanently arrests cells in replicative senescence. It has been proposed that such short telomeres are not subjected to fusion events, possibly because they retain sufficient shelterin complex to inhibit them [131]. However, inactivation of the checkpoint allows these cells to bypass the senescent state, reaching a second proliferative barrier, known as telomere crisis, in which critically short telomeres become vulnerable to fusion events and formation of dicentric chromosomes [132,133]. Although most cells die, the few cells that re-elongate their telomeres proliferate indefinitely. Telomere shortening and activation of the DNA damage checkpoint occur also in ageing post-mitotic cells, including cardiomyocytes, adipocytes, neurons, osteocytes, and osteoblasts [134], suggesting that cellular senescence contributes to organismal ageing.

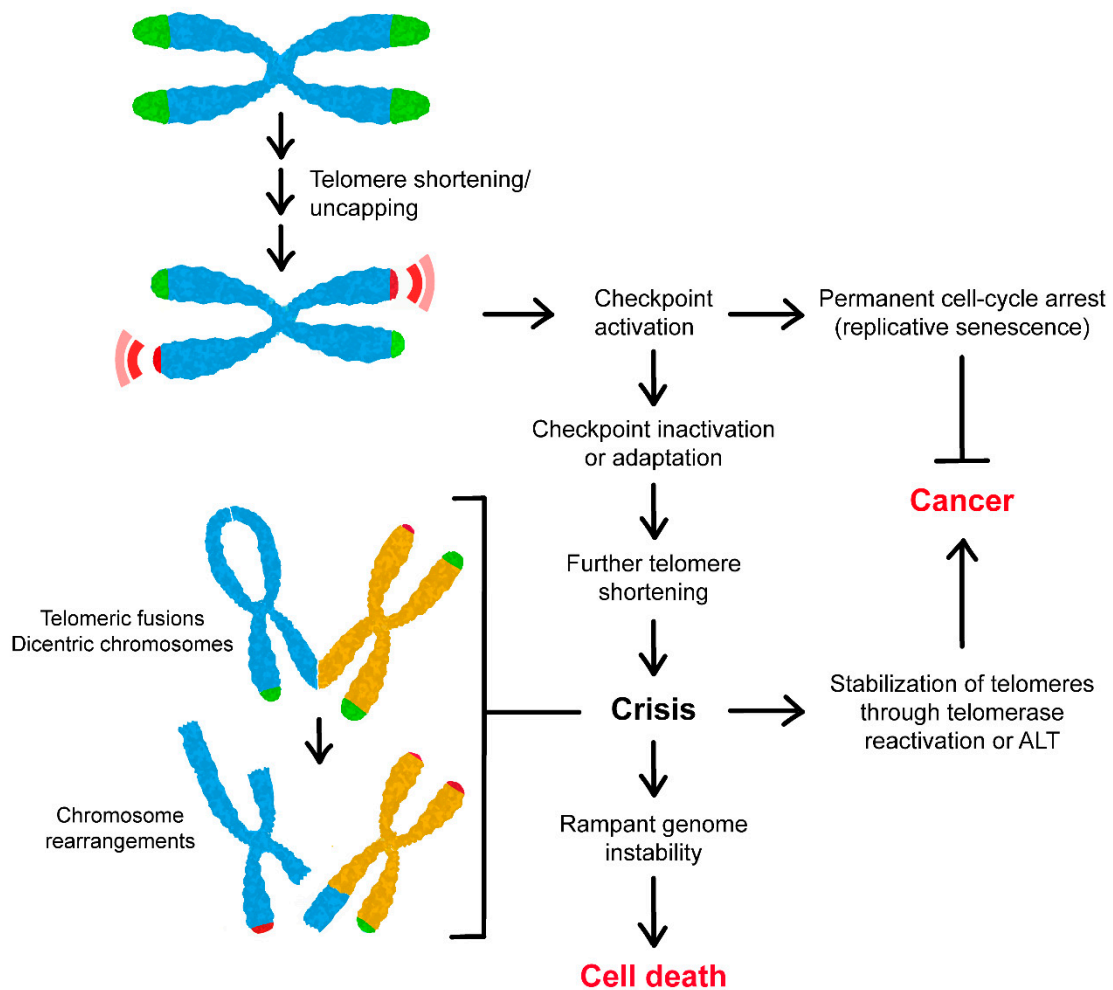


Figure 5. Telomere attrition in cancer. Telomere attrition due to either deficiency in capping proteins or loss of telomeric DNA induces a DNA damage checkpoint response that leads to a permanent cell-cycle arrest and entry into a non-dividing state called replicative senescence, which provides a potent anticancer barrier. However, checkpoint inactivation through mutations or adaptation allows cells to bypass senescence, reaching a second proliferative state, known as telomere crisis, during which critically short telomeres become vulnerable to end-to-end fusions, forming dicentric chromosomes and resulting in deletions, amplifications, and translocations. This rampant genomic instability leads to death of most cells, but the few cells that re-stabilize telomeres by re-activating telomerase or inducing ALT can survive and proliferate indefinitely, thus promoting malignant transformation.

Telomerase-negative *S. cerevisiae* cells, where a single telomere was engineered to be reduced in length without affecting the integrity of its tip, accelerate the onset of senescence [135,136]. This signaling telomere is bound by RPA, Rad52, Ddc2, and Mec1 repair and checkpoint proteins, indicating that a single very short telomere is sufficient to induce checkpoint activation and replicative senescence in yeast. Similarly, the presence of few very short telomeres in mouse cells is sufficient to trigger replicative senescence [137], indicating that is not the average but rather the presence of one or few critically short telomeres that causes checkpoint activation and senescence entry. Importantly, Mec1 is required to induce a senescent state in the presence of a critically short telomere [135], suggesting that ssDNA is the primary signal triggering senescence. Consistent with this hypothesis, 5'-3' resection is stimulated at short telomeres that expose subtelomeric ssDNA [138]. Furthermore, the lack of telomere-processing proteins, such as MRX, delays senescence, while the lack of MRX inhibitors, such as Rif2, anticipates it [139].

The absence of Tel1 also delays senescence [126,135,139,140]. As Tel1 promotes ssDNA generation at both DSBs and telomeres [141], the delayed senescence in Tel1-deficient telomerase-negative cells can be due to a reduced amount of telomeric ssDNA. By studying the senescence phenotype of telomerase-deficient cells lacking Tel1 or expressing the hyperactive Tel1-hy184 mutant variant, which has been identified because of its ability to compensate for the lack of Mec1 function [142], it was shown that Tel1-hy184 anticipates senescence, while the lack of Tel1 or of its kinase activity delays it [143]. Neither Tel1-hy184 nor Tel1 kinase defective variant affects the generation of ssDNA at telomeres, suggesting that Tel1 function in promoting senescence is not directly linked to ssDNA generation. The finding that the anticipated senescence triggered by Tel1-hy184 completely depends on Rad9 and only partially on Mec1 suggests that Tel1 promotes senescence mainly by directly signaling the presence of dysfunctional telomeres to a Rad9-dependent checkpoint. These results suggest that, as telomeres shorten in the absence of telomerase and the negative control exerted by Rif2 on MRX-Tel1 activity declines, MRX recruits Tel1 at telomeres that can directly signal to the checkpoint machinery.

5. Escape from Telomere-Induced Replicative Senescence

Replicative senescence elicited by activation of the checkpoint response is a state of stable, terminal cell-cycle arrest that acts as a barrier against tumorigenesis. Importantly, telomere attrition in ageing telomerase-deficient mice lacking the checkpoint protein p53 was reported to cause epithelial cancers by a process of breakage-fusion-bridge [144], indicating that the status of the checkpoint response dictates whether the short telomeres promote or suppress cancer.

The checkpoint response can be overcome either through mutational inactivation of its components or through adaptation, which is a phenomenon originally described in yeast as the ability of cells to overcome a sustained checkpoint arrest despite the presence of unrepaired DNA damage. The ability of cells to adapt to altered telomere length or structure has been first observed in *S. cerevisiae* by Sandell and Zakian, who discovered that, after elimination of a single telomere and checkpoint activation, many cells were capable to resume cell-cycle progression without having repaired the damaged chromosome [3]. In budding yeast, adaptation has been observed also in response to a single unreparable DSB [145–147], where it requires the polo kinase Cdc5 [145], the phosphatase Ptc2 [148], the regulatory subunits Ckb1 and Ckb2 of casein kinase II (CKII) [145], and the recombination proteins Tid1 and Srs2 [149]. During adaptation, Rad53 checkpoint kinase is inactivated [147], thus allowing cells to resume cell-cycle progression to get an opportunity to repair DNA damage in other cell-cycle phases [150,151]. Mechanisms to abrogate a prolonged checkpoint arrest were also reported in *Xenopus laevis* and human cells and a similar genetic requirement suggests a common evolutionary origin [152,153].

Budding yeast cells are capable to adapt to the checkpoint that is elicited not only in response to loss of telomerase but also to capping defects. In fact, downregulation of the checkpoint response can allow *cdc13* mutant cells to adapt to the presence of uncapped telomeres and resume cell-cycle progression [145,154]. In these cells, uncapped telomeres persist throughout the cell cycle and are bound by DNA repair proteins, indicating that the resuming of cell-cycle progression is not due to repair of damaged telomeres but to abrogation of the checkpoint response. Proteins, such as Tid1, Ptc2, and Cdc5, known to be required for adaptation to a single unrepaired DSB, are required to allow adaptation of *cdc13* mutant cells [154], arguing that adaptation to a DSB or to capping defects occurs by a similar mechanism.

By tracking individual cell lineages over time using a microfluidic-based approach coupled to single-cell imaging, it was found that adaptation-deficient cells have a higher mutation rate than adaptation-proficient cells [155], indicating that adaptation contributes to genome instability. This genome instability can be due to end-to-end telomeric fusions to form dicentric chromosomes and subsequent breakage-fusion-bridge cycles [156]. The increased genome instability in adapted cells might be due also to the use of mutagenic

repair pathways, such as NHEJ in G1 or microhomology-mediated end-joining, that were not utilized during a checkpoint-mediated cell-cycle arrest [150].

The rampant chromosome instability experienced by adapted cells has important implications for understanding the early steps of tumorigenesis, during which precancerous cells undergo a phase of high genome instability [155]. Although cells undergoing checkpoint adaptation almost die in the subsequent cell cycles due to high levels of genetic instability, cells that overcome this crisis by re-stabilizing critically shortened telomeres can proliferate indefinitely. Consistent with this model, ectopic expression of TERT in combination with two oncogenes was shown to promote malignant transformation of primary human cells [157]. Thus, the chain of events of telomeric alterations, checkpoint activation, and adaptation is a major mechanism that enables malignant transformation in cells lacking telomerase activity (Figure 5).

6. Telomerase-Independent Re-Stabilization of Telomere Length

Although reactivation of telomerase is the most common telomere maintenance mechanism in cancer, cancer cells can use telomerase-independent recombination-based mechanisms, called alternative lengthening of telomeres (ALT), to re-elongate their telomeres. ALT was discovered by Lundblad and Blackburn, who found that yeast cells lacking telomerase can evade the senescence state by re-elongating their telomeres through the use of recombination [158]. Cells that restabilize telomeres by activating such mechanisms have been called post-senescence survivors. Based on telomere organization and genetic requirements, two types of survivors can be described [158–160], although other mechanisms can contribute to stabilize telomeres in the absence of telomerase [161–164]. *S. cerevisiae* telomeres are comprised of ~300 bp of double-stranded TG₁₋₃/C₁₋₃A repeats with a 3'-ended 8–15 nucleotides overhang [165,166]. Internal to the TG₁₋₃/C₁₋₃A tracts are repetitive DNA elements, called X and Y'. Type I survivors arise through amplification of the subtelomeric Y' sequences that may stem from non-reciprocal translocations or integration of extra-chromosomal Y' circles into the short telomeres. By contrast, type II survivors harbor long and heterogeneous telomeric repeat tracts with no rearrangement of Y' elements. Furthermore, type I requires Rad51, whereas type II relies on Rad50, Rad59, and Sgs1 [160,167–170]. Both types instead depend on the recombination protein Rad52 and the nonessential DNA polymerase δ subunit Pol32. The requirement for Pol32 suggests that break-induced replication (BIR), which is a mechanism used to repair one-ended DSBs through strand invasion into a homologous donor sequence, can be involved [171]. However, a recent analysis of ultra-long sequencing of chromosome ends has revealed that survivors contain DNA sequences that can be attributed to both type I and II [172]. Based on this finding, it was proposed a unified pathway that comprises two sequential steps: formation of precursors by Rad51-mediated strand invasion, followed by maturation into survivors via a Rad59-dependent pathway. In any case, reactivation of telomerase in such cells leads to reversal of the senescence phenotype and restores a telomerase-mediated mode of telomere elongation [173,174], indicating that telomerase represses recombination at telomeres.

The absence of telomerase results in replicative senescence also in *S. pombe* cells that can escape it by maintaining telomeres in a telomerase-independent mode [175]. However, most survivors are formed by circularization of each individual chromosome, possibly because loss of protection due to telomere shortening allows end-to-end fusions to occur [175–177]. Interestingly, a new type of survivor, termed HAATI (heterochromatin amplification-mediated and telomerase-independent), that relies on the presence of non-telomeric heterochromatin, has been described in *S. pombe* [178].

ALT mechanisms that depend on homologous recombination can be observed also in human telomerase-negative immortalized cell lines and in 10–15% of human cancers, which use them to re-elongate their telomeres and gain unlimited proliferative potential [179,180]. A plasmid tag inserted into a single telomere in mouse or human ALT cells was found to be copied to other telomeres or duplicated in its original location without the involvement of

other telomeres [181–183]. This finding suggests that a telomere can use itself or a telomere on a sister chromatid or on another chromosome as a copy template by BIR. Interestingly, the BIR mechanism has been proposed to be responsible also for the generation of type II survivors in yeast [171].

The cause of the triggering of HR-based mechanisms at telomeres remains poorly understood. As stated above, telomeres are intrinsic obstacles for replication fork progression in both yeast [74,75] and mammals [184], because of the presence of telomeric DNA-bound proteins and DNA secondary structures. Interestingly, in yeast, phosphorylation of Rad53 upon inactivation of telomerase or of Cdc13 depends not only on Rad9 but also on Mrc1 [185,186], a checkpoint protein implicated in the response to replication stress [53,56]. Furthermore, Mrc1 was found to be phosphorylated during senescence [186], suggesting that replication stresses occur at short telomeres. Bidimensional gels assessing replication intermediates in telomerase-negative fission and budding yeast cells revealed a severe impairment of telomere replication that correlates with an accumulation of four-branch DNA structures [187,188]. Furthermore, using a reconstituted replication assay, it has been shown that budding yeast Rap1 acts as a roadblock to the replisome and potently inhibits lagging strand replication behind the fork [74]. These data support the idea that the absence of telomerase leads to replication stresses at telomeres that force cells to use recombination to repair damaged telomeres and maintain viability.

In human cells, commitment to ALT is often associated with loss of ATRX/DAXX chromatin remodeling complex, changes in telomeric chromatin, or formation of RNA:DNA hybrids [189,190]. In particular, loss of ATRX causes decompaction/alterations of telomeric chromatin and increased replication stress [191–194], suggesting that altered telomeric chromatin can drive ALT by inducing replication stress that generates substrates for the recombination machinery [195]. Fork progression can be hampered also by transcription from subtelomeric and telomeric regions that can generate RNA:DNA hybrids, which are structures formed by the annealing of nascent RNA transcripts to the DNA template strand. The best known RNA species that are transcribed from the subtelomeric region toward the chromosome end are long non-coding RNAs conserved in many species that are called TERRA (telomeric repeat-containing RNAs). Interestingly, telomere shortening in both *S. cerevisiae* and *S. pombe* induces TERRA transcription [196–199], which leads to the generation of RNA:DNA hybrids and an increased frequency of telomere recombination in the absence of telomerase [200–202]. Moreover, inhibition of TERRA transcription decreases DNA replication stress and DNA damage at telomeres, and impairs ALT activity and telomere length maintenance by BIR [203].

7. Conclusions

Telomere maintenance, which is essential for chromosome integrity, presents multiple challenges. Since the pioneering work of Muller and McClintock, extensive research has revealed that telomeric DNA is bound by a growing list of proteins that serve to regulate its length and protect it from unwarranted fusion, recombination, and degradation events. These results imply that genomes are not uniformly repairable and that some genomic loci, such as telomeric DNA, resist DNA repair. This irreparability may be the consequence of their functions in ensuring the maintenance of linear chromosomes.

Inactivation of telomerase via genetic manipulation in budding yeast recapitulates the process of telomere shortening and induction of replicative senescence observed in human somatic cells. Furthermore, budding yeast uses strategies to escape senescence that resemble those used by human cells. As telomerase removal in budding yeast can allow detection of early and rare events, this organism can be used to decipher the causes and consequences of replication stress at telomeres, the molecular events leading to induction of recombination and telomere-driven mutagenesis. Therefore, the data obtained with this organism can help investigations in mammalian models to make rapid progress in this field that is important for human health.

As precancerous cells undergo rapid proliferation that leads to telomere shortening, it is also clear that, in certain types of cancer, telomere attrition can be a promoter of tumorigenesis. The role of telomere in malignancy and metastasis has been proved in telomerase-deficient checkpoint-mutated mice, in which telomere attrition promotes the development of epithelial cancers by a process of fusion-bridge breakage that leads to the formation of complex non-reciprocal translocations [144,204]. Furthermore, reactivation of telomerase in tumor cells that have already experienced telomere dysfunction is sufficient to dampen the checkpoint response and quell rampant chromosome instability, enabling full malignant transformation [205]. The increased telomerase activity observed in most cancers has led to the development of several strategies to target TERT. However, as TERT inhibition was found to select activation of ALT pathways in lymphoma [206], combined drugs that suppress telomerase and ALT-pathway could be beneficial to minimize emergence of resistance.

Author Contributions: Conceptualization, M.P.L.; writing—original draft, M.P.L., E.C., and M.G.; writing—review and editing C.R., P.P., C.V.C. and D.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Fondazione AIRC, IG 2017–ID. 19783 to M.P.L. and Progetti di Ricerca di Interesse Nazionale (PRIN) 2017 to M.P.L. and D.B.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Muller, H.J. The remaking of chromosomes. *Collect. Net* **1938**, *13*, 181–198.
2. McClintock, B. The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. *Genetics* **1938**, *23*, 315–376. [[CrossRef](#)] [[PubMed](#)]
3. Sandell, L.L.; Zakian, V.A. Loss of a yeast telomere: Arrest, recovery, and chromosome loss. *Cell* **1993**, *75*, 729–739. [[CrossRef](#)]
4. Blackburn, E.H.; Gall, J.G. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in *Tetrahymena*. *J. Mol. Biol.* **1978**, *120*, 33–53. [[CrossRef](#)]
5. Klobutcher, L.A.; Swanton, M.T.; Donini, P.; Prescott, D.M. All gene-sized DNA molecules in four species of hypotrichs have the same terminal sequence and an unusual 3' terminus. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 3015–3019. [[CrossRef](#)]
6. Szostak, J.W.; Blackburn, E.H. Cloning yeast telomeres on linear plasmid vectors. *Cell* **1982**, *29*, 245–255. [[CrossRef](#)]
7. Shampay, J.; Szostak, J.W.; Blackburn, E.H. DNA sequences of telomeres maintained in yeast. *Nature* **1984**, *310*, 154–157. [[CrossRef](#)] [[PubMed](#)]
8. Greider, C.W.; Blackburn, E.H. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* **1985**, *43*, 405–413. [[CrossRef](#)]
9. Greider, C.W.; Blackburn, E.H. The telomere terminal transferase of *Tetrahymena* is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* **1987**, *51*, 887–898. [[CrossRef](#)]
10. Greider, C.W.; Blackburn, E.H. A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. *Nature* **1989**, *337*, 331–337. [[CrossRef](#)] [[PubMed](#)]
11. Yu, G.L.; Bradley, J.D.; Attardi, L.D.; Blackburn, E.H. In vivo alteration of telomere sequences and senescence caused by mutated *Tetrahymena* telomerase RNAs. *Nature* **1990**, *344*, 126–132. [[CrossRef](#)] [[PubMed](#)]
12. Lundblad, V.; Szostak, J.W. A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell* **1989**, *57*, 633–643. [[CrossRef](#)]
13. Lendvay, T.S.; Morris, D.K.; Sah, J.; Balasubramanian, B.; Lundblad, V. Senescence mutants of *Saccharomyces cerevisiae* with a defect in telomere replication identify three additional EST genes. *Genetics* **1996**, *144*, 1399–1412. [[CrossRef](#)] [[PubMed](#)]
14. Singer, M.S.; Gottschling, D.E. TLC1: Template RNA component of *Saccharomyces cerevisiae* telomerase. *Science* **1994**, *266*, 404–409. [[CrossRef](#)]
15. Lingner, J.; Hughes, T.R.; Shevchenko, A.; Mann, M.; Lundblad, V.; Cech, T.R. Reverse transcriptase motifs in the catalytic subunit of telomerase. *Science* **1997**, *276*, 561–567. [[CrossRef](#)]
16. Feng, J.; Funk, W.D.; Wang, S.S.; Weinrich, S.L.; Avilion, A.A.; Chiu, C.P.; Adams, R.R.; Chang, E.; Allsopp, R.C.; Yu, J.; et al. The RNA component of human telomerase. *Science* **1995**, *269*, 1236–1241. [[CrossRef](#)] [[PubMed](#)]

17. Harrington, L.; McPhail, T.; Mar, V.; Zhou, W.; Oulton, R.; Bass, M.B.; Arruda, I.; Robinson, M.O. A mammalian telomerase-associated protein. *Science* **1997**, *275*, 973–977. [[CrossRef](#)]
18. Waterman, D.P.; Haber, J.E.; Smolka, M.B. Checkpoint responses to DNA double-strand breaks. *Annu. Rev. Biochem.* **2020**, *89*, 103–133. [[CrossRef](#)]
19. Chang, H.H.Y.; Pannunzio, N.R.; Adachi, N.; Lieber, M.R. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 495–506. [[CrossRef](#)]
20. Mimitou, E.P.; Symington, L.S. Ku prevents Exo1 and Sgs1-dependent resection of DNA ends in the absence of a functional MRX complex or Sae2. *EMBO J.* **2010**, *29*, 3358–3369. [[CrossRef](#)]
21. Shim, E.Y.; Chung, W.H.; Nicolette, M.L.; Zhang, Y.; Davis, M.; Zhu, Z.; Paull, T.T.; Ira, G.; Lee, S.E. *Saccharomyces cerevisiae* Mre11/Rad50/Xrs2 and Ku proteins regulate association of Exo1 and Dna2 with DNA breaks. *EMBO J.* **2010**, *29*, 3370–3380. [[CrossRef](#)] [[PubMed](#)]
22. Mehta, A.; Haber, J.E. Sources of DNA double-strand breaks and models of recombinational DNA repair. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, a016428. [[CrossRef](#)]
23. Cejka, P.; Symington, L.S. DNA end resection: Mechanism and control. *Annu. Rev. Genet.* **2021**, *55*, 285–307. [[CrossRef](#)] [[PubMed](#)]
24. Syed, A.; Tainer, J.A. The MRE11-RAD50-NBS1 complex conducts the orchestration of damage signaling and outcomes to stress in DNA replication and repair. *Annu. Rev. Biochem.* **2018**, *87*, 263–294. [[CrossRef](#)] [[PubMed](#)]
25. Paull, T.T.; Gellert, M. The 3' to 5' exonuclease activity of Mre11 facilitates repair of DNA double-strand breaks. *Mol. Cell* **1998**, *1*, 969–979. [[CrossRef](#)]
26. Trujillo, K.M.; Yuan, S.S.; Lee, E.Y.; Sung, P. Nuclease activities in a complex of human recombination and DNA repair factors Rad50, Mre11, and p95. *J. Biol. Chem.* **1998**, *273*, 21447–21450. [[CrossRef](#)]
27. Cannavo, E.; Cejka, P. Sae2 promotes dsDNA endonuclease activity within Mre11-Rad50-Xrs2 to resect DNA breaks. *Nature* **2014**, *514*, 122–125. [[CrossRef](#)]
28. Mimitou, E.P.; Symington, L.S. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* **2008**, *455*, 770–774. [[CrossRef](#)]
29. Zhu, Z.; Chung, W.H.; Shim, E.Y.; Lee, S.E.; Ira, G. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* **2008**, *134*, 981–994. [[CrossRef](#)]
30. Cejka, P.; Cannavo, E.; Polaczek, P.; Masuda-Sasa, T.; Pokharel, S.; Campbell, J.L.; Kowalczykowski, S.C. DNA end resection by Dna2-Sgs1-RPA and its stimulation by Top3-Rmi1 and Mre11-Rad50-Xrs2. *Nature* **2010**, *467*, 112–116. [[CrossRef](#)]
31. Niu, H.; Chung, W.H.; Zhu, Z.; Kwon, Y.; Zhao, W.; Chi, P.; Prakash, R.; Seong, C.; Liu, D.; Lu, L.; et al. Mechanism of the ATP-dependent DNA end-resection machinery from *Saccharomyces cerevisiae*. *Nature* **2010**, *467*, 108–111. [[CrossRef](#)] [[PubMed](#)]
32. Garcia, V.; Phelps, S.E.; Gray, S.; Neale, M.J. Bidirectional resection of DNA double-strand breaks by Mre11 and Exo1. *Nature* **2011**, *479*, 241–244. [[CrossRef](#)] [[PubMed](#)]
33. Shibata, A.; Moiani, D.; Arvai, A.S.; Perry, J.; Harding, S.M.; Genois, M.M.; Maity, R.; van Rossum-Fikkert, S.; Kertokallio, A.; Romoli, F.; et al. DNA double-strand break repair pathway choice is directed by distinct MRE11 nuclease activities. *Mol. Cell* **2014**, *53*, 7–18. [[CrossRef](#)] [[PubMed](#)]
34. Reginato, G.; Cannavo, E.; Cejka, P. Physiological protein blocks direct the Mre11-Rad50-Xrs2 and Sae2 nuclease complex to initiate DNA end resection. *Genes Dev.* **2017**, *31*, 2325–2330. [[CrossRef](#)]
35. Wang, W.; Daley, J.M.; Kwon, Y.; Krasner, D.S.; Sung, P. Plasticity of the Mre11-Rad50-Xrs2-Sae2 nuclease ensemble in the processing of DNA-bound obstacles. *Genes Dev.* **2017**, *31*, 2331–2336. [[CrossRef](#)]
36. Lammens, K.; Bemeleit, D.J.; Möckel, C.; Clausing, E.; Schele, A.; Hartung, S.; Schiller, C.B.; Lucas, M.; Angermüller, C.; Söding, J.; et al. The Mre11:Rad50 structure shows an ATP-dependent molecular clamp in DNA double-strand break repair. *Cell* **2011**, *145*, 54–66. [[CrossRef](#)]
37. Williams, G.J.; Williams, R.S.; Williams, J.S.; Moncalian, G.; Arvai, A.S.; Limbo, O.; Guenther, G.; SilDas, S.; Hammel, M.; Russell, P.; et al. ABC ATPase signature helices in Rad50 link nucleotide state to Mre11 interface for DNA repair. *Nat. Struct. Mol. Biol.* **2011**, *18*, 423–431. [[CrossRef](#)]
38. Möckel, C.; Lammens, K.; Schele, A.; Hopfner, K.P. ATP driven structural changes of the bacterial Mre11:Rad50 catalytic head complex. *Nucleic Acids Res.* **2012**, *40*, 914–927. [[CrossRef](#)]
39. Deshpande, R.A.; Williams, G.J.; Limbo, O.; Williams, R.S.; Kuhnlein, J.; Lee, J.H.; Classen, S.; Guenther, G.; Russell, P.; Tainer, J.A.; et al. ATP-driven Rad50 conformations regulate DNA tethering, end resection, and ATM checkpoint signaling. *EMBO J.* **2014**, *33*, 482–500. [[CrossRef](#)]
40. Käshammer, L.; Saathoff, J.H.; Lammens, K.; Gut, F.; Bartho, J.; Alt, A.; Kessler, B.; Hopfner, K.P. Mechanism of DNA end sensing and processing by the Mre11-Rad50 complex. *Mol. Cell* **2019**, *76*, 382–394. [[CrossRef](#)]
41. Lustig, A.J.; Petes, T.D. Identification of yeast mutants with altered telomere structure. *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 1398–1402. [[CrossRef](#)] [[PubMed](#)]
42. Nakada, D.; Matsumoto, K.; Sugimoto, K. ATM-related Tel1 associates with double-strand breaks through an Xrs2-dependent mechanism. *Genes Dev.* **2003**, *17*, 1957–1962. [[CrossRef](#)] [[PubMed](#)]
43. Uziel, T.; Lerenthal, Y.; Moyal, L.; Andegeko, Y.; Mittelman, L.; Shiloh, Y. Requirement of the MRN complex for ATM activation by DNA damage. *EMBO J.* **2003**, *22*, 5612–5621. [[CrossRef](#)] [[PubMed](#)]

44. Lee, J.H.; Paull, T.T. Direct activation of the ATM protein kinase by the Mre11/Rad50/Nbs1 complex. *Science* **2004**, *304*, 93–96. [[CrossRef](#)]
45. Lee, J.H.; Paull, T.T. ATM activation by DNA double-strand breaks through the Mre11-Rad50-Nbs1 complex. *Science* **2005**, *308*, 551–554. [[CrossRef](#)]
46. You, Z.; Chahwan, C.; Bailis, J.; Hunter, T.; Russell, P. ATM activation and its recruitment to damaged DNA require binding to the C terminus of Nbs1. *Mol. Cell Biol.* **2005**, *25*, 5363–5379. [[CrossRef](#)]
47. Cassani, C.; Gobbin, E.; Wang, W.; Niu, H.; Clerici, M.; Sung, P.; Longhese, M.P. Tel1 and Rif2 regulate MRX functions in end-tethering and repair of DNA double-strand breaks. *PLoS Biol.* **2016**, *14*, e1002387. [[CrossRef](#)]
48. Zou, L.; Elledge, S.J. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* **2003**, *300*, 1542–1548. [[CrossRef](#)]
49. Rouse, J.; Jackson, S.P. Lcd1p recruits Mec1p to DNA lesions in vitro and in vivo. *Mol. Cell* **2002**, *9*, 857–869. [[CrossRef](#)]
50. Sanchez, Y.; Bachant, J.; Wang, H.; Hu, F.; Liu, D.; Tetzlaff, M.; Elledge, S.J. Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms. *Science* **1999**, *286*, 1166–1171. [[CrossRef](#)]
51. Gilbert, C.S.; Green, C.M.; Lowndes, N.F. Budding yeast Rad9 is an ATP-dependent Rad53 activating machine. *Mol. Cell* **2001**, *8*, 129–136. [[CrossRef](#)]
52. Sweeney, F.D.; Yang, F.; Chi, A.; Shabanowitz, J.; Hunt, D.F.; Durocher, D. *Saccharomyces cerevisiae* Rad9 acts as a Mec1 adaptor to allow Rad53 activation. *Curr. Biol.* **2005**, *15*, 1364–1375. [[CrossRef](#)] [[PubMed](#)]
53. Alcasabas, A.A.; Osborn, A.J.; Bachant, J.; Hu, F.; Werler, P.J.; Bousset, K.; Furuya, K.; Diffley, J.F.; Carr, A.M.; Elledge, S.J. Mrc1 transduces signals of DNA replication stress to activate Rad53. *Nat. Cell Biol.* **2001**, *3*, 958–965. [[CrossRef](#)]
54. Tanaka, K.; Russell, P. Mrc1 channels the DNA replication arrest signal to checkpoint kinase Cds1. *Nat. Cell Biol.* **2001**, *3*, 966–972. [[CrossRef](#)] [[PubMed](#)]
55. Katou, Y.; Kanoh, Y.; Bando, M.; Noguchi, H.; Tanaka, H.; Ashikari, T.; Sugimoto, K.; Shirahige, K. S-phase checkpoint proteins Tof1 and Mrc1 form a stable replication-pausing complex. *Nature* **2003**, *424*, 1078–1083. [[CrossRef](#)] [[PubMed](#)]
56. Osborn, A.J.; Elledge, S.J. Mrc1 is a replication fork component whose phosphorylation in response to DNA replication stress activates Rad53. *Genes Dev.* **2003**, *17*, 1755–1767. [[CrossRef](#)]
57. Michelson, R.J.; Rosenstein, S.; Weinert, T. A telomeric repeat sequence adjacent to a DNA double-stranded break produces an antieckpoint. *Genes Dev.* **2005**, *19*, 2546–2559. [[CrossRef](#)]
58. Ribeyre, C.; Shore, D. Antieckpoint pathways at telomeres in yeast. *Nat. Struct. Mol. Biol.* **2012**, *19*, 307–313. [[CrossRef](#)] [[PubMed](#)]
59. Fumagalli, M.; Rossiello, F.; Clerici, M.; Barozzi, S.; Cittaro, D.; Kaplunov, J.M.; Bucci, G.; Dobrev, M.; Matti, V.; Beausejour, C.M.; et al. Telomeric DNA damage is irreparable and causes persistent DNA-damage-response activation. *Nat. Cell Biol.* **2012**, *14*, 355–365. [[CrossRef](#)] [[PubMed](#)]
60. Weinert, T.A.; Hartwell, L.H. The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* **1988**, *241*, 317–322. [[CrossRef](#)] [[PubMed](#)]
61. Garvik, B.; Carson, M.; Hartwell, L. Single-stranded DNA arising at telomeres in *cdc13* mutants may constitute a specific signal for the *RAD9* checkpoint. *Mol. Cell Biol.* **1995**, *15*, 6128–6138. [[CrossRef](#)]
62. Grandin, N.; Reed, S.I.; Charbonneau, M. Stn1, a new *Saccharomyces cerevisiae* protein, is implicated in telomere size regulation in association with Cdc13. *Genes Dev.* **1997**, *11*, 512–527. [[CrossRef](#)] [[PubMed](#)]
63. Grandin, N.; Damon, C.; Charbonneau, M. Ten1 functions in telomere end protection and length regulation in association with Stn1 and Cdc13. *EMBO J.* **2001**, *20*, 1173–1183. [[CrossRef](#)]
64. Xu, L.; Petreaca, R.C.; Gasparyan, H.J.; Vu, S.; Nugent, C.I. TEN1 is essential for CDC13-mediated telomere capping. *Genetics* **2009**, *183*, 793–810. [[CrossRef](#)] [[PubMed](#)]
65. Price, C.M.; Boltz, K.A.; Chaiken, M.F.; Stewart, J.A.; Beilstein, M.A.; Shippen, D.E. Evolution of CST function in telomere maintenance. *Cell Cycle* **2010**, *9*, 3157–3165. [[CrossRef](#)] [[PubMed](#)]
66. Gao, H.; Cervantes, R.B.; Mandell, E.K.; Otero, J.H.; Lundblad, V. RPA-like proteins mediate yeast telomere function. *Nat. Struct. Mol. Biol.* **2007**, *14*, 208–214. [[CrossRef](#)] [[PubMed](#)]
67. 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. [[CrossRef](#)] [[PubMed](#)]
68. Vodenicharov, M.D.; Laterreur, N.; Wellinger, R.J. Telomere capping in non-dividing yeast cells requires Yku and Rap1. *EMBO J.* **2010**, *29*, 3007–3019. [[CrossRef](#)] [[PubMed](#)]
69. Vodenicharov, M.D.; Wellinger, R.J. DNA degradation at unprotected telomeres in yeast is regulated by the CDK1 (Cdc28/Clb) cell-cycle kinase. *Mol. Cell* **2006**, *24*, 127–137. [[CrossRef](#)] [[PubMed](#)]
70. Langston, R.E.; Palazzola, D.; Bonnell, E.; Wellinger, R.J.; Weinert, T. Loss of Cdc13 causes genome instability by a deficiency in replication-dependent telomere capping. *PLoS Genet.* **2020**, *16*, e1008733. [[CrossRef](#)] [[PubMed](#)]
71. Diede, S.J.; Gottschling, D.E. Telomerase-mediated telomere addition in vivo requires DNA primase and DNA polymerases alpha and delta. *Cell* **1999**, *99*, 723–733. [[CrossRef](#)]
72. Qi, H.; Zakian, V.A. The *Saccharomyces* telomere-binding protein Cdc13p interacts with both the catalytic subunit of DNA polymerase alpha and the telomerase-associated est1 protein. *Genes Dev.* **2000**, *14*, 1777–1788. [[CrossRef](#)]

73. Grossi, S.; Puglisi, A.; Dmitriev, P.V.; Lopes, M.; Shore, D. Pol12, the B subunit of DNA polymerase alpha, functions in both telomere capping and length regulation. *Genes Dev.* **2004**, *18*, 992–1006. [[CrossRef](#)]
74. Douglas, M.E.; Diffley, J.F.X. Budding yeast Rap1, but not telomeric DNA, is inhibitory for multiple stages of DNA replication in vitro. *Nucleic Acids Res.* **2021**, *49*, 5671–5683. [[CrossRef](#)] [[PubMed](#)]
75. Makovets, S.; Herskowitz, I.; Blackburn, E.H. Anatomy and dynamics of DNA replication fork movement in yeast telomeric regions. *Mol. Cell Biol.* **2004**, *24*, 4019–4031. [[CrossRef](#)] [[PubMed](#)]
76. Gu, P.; Min, J.N.; Wang, Y.; Huang, C.; Peng, T.; Chai, W.; Chang, S. CTC1 deletion results in defective telomere replication, leading to catastrophic telomere loss and stem cell exhaustion. *EMBO J.* **2012**, *31*, 2309–2321. [[CrossRef](#)] [[PubMed](#)]
77. Wang, F.; Stewart, J.A.; Kasbek, C.; Zhao, Y.; Wright, W.E.; Price, C.M. Human CST has independent functions during telomere duplex replication and C-strand fill-in. *Cell Rep.* **2012**, *2*, 1096–1103. [[CrossRef](#)] [[PubMed](#)]
78. Feng, X.; Hsu, S.J.; Kasbek, C.; Chaiken, M.; Price, C.M. CTC1-mediated C-strand fill-in is an essential step in telomere length maintenance. *Nucleic Acids Res.* **2017**, *45*, 4281–4293. [[CrossRef](#)] [[PubMed](#)]
79. Wu, P.; Takai, H.; de Lange, T. Telomeric 3' overhangs derive from resection by Exo1 and Apollo and fill-in by POT1b-associated CST. *Cell* **2012**, *150*, 39–52. [[CrossRef](#)] [[PubMed](#)]
80. Mirman, Z.; Lottersberger, F.; Takai, H.; Kibe, T.; Gong, Y.; Takai, K.; Bianchi, A.; Zimmermann, M.; Durocher, D.; de Lange, T. 53BP1-RIF1-shieldin counteracts DSB resection through CST- and Pol α -dependent fill-in. *Nature* **2018**, *560*, 112–116. [[CrossRef](#)]
81. Lyu, X.; Sang, P.B.; Chai, W. CST in maintaining genome stability: Beyond telomeres. *DNA Repair* **2021**, *102*, 103104. [[CrossRef](#)] [[PubMed](#)]
82. Gravel, S.; Larrivee, M.; Labrecque, P.; Wellinger, R.J. Yeast Ku as a regulator of chromosomal DNA end structure. *Science* **1998**, *280*, 741–744. [[CrossRef](#)] [[PubMed](#)]
83. Maringele, L.; Lydall, D. EXO1-dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast *yku70Delta* mutants. *Genes Dev.* **2002**, *16*, 1919–1933. [[CrossRef](#)] [[PubMed](#)]
84. Bonetti, D.; Clerici, M.; Anbalagan, S.; Martina, M.; Lucchini, G.; Longhese, M.P. Shelterin-like proteins and Yku inhibit nucleolytic processing of *Saccharomyces cerevisiae* telomeres. *PLoS Genet.* **2010**, *6*, e1000966. [[CrossRef](#)]
85. Teo, S.H.; Jackson, S.P. Telomerase subunit overexpression suppresses telomere-specific checkpoint activation in the yeast *yku80* mutant. *EMBO Rep.* **2001**, *2*, 197–202. [[CrossRef](#)]
86. Holland, C.L.; Sanderson, B.A.; Titus, J.K.; Weis, M.F.; Riojas, A.M.; Malczewskyj, E.; Wasko, B.M.; Lewis, L.K. Suppression of telomere capping defects of *Saccharomyces cerevisiae yku70* and *yku80* mutants by telomerase. *G3* **2021**, *11*, jkab359. [[CrossRef](#)]
87. Wang, Y.; Ghosh, G.; Hendrickson, E.A. Ku86 represses lethal telomere deletion events in human somatic cells. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 12430–12435. [[CrossRef](#)]
88. Palm, W.; Hockemeyer, D.; Kibe, T.; de Lange, T. Functional dissection of human and mouse POT1 proteins. *Mol. Cell Biol.* **2009**, *29*, 471–482. [[CrossRef](#)]
89. Sfeir, A.; Kabir, S.; van Overbeek, M.; Celli, G.B.; de Lange, T. Loss of Rap1 induces telomere recombination in the absence of NHEJ or a DNA damage signal. *Science* **2010**, *327*, 1657–1661. [[CrossRef](#)]
90. Lustig, A.J.; Kurtz, S.; Shore, D. Involvement of the silencer and UAS binding protein RAP1 in regulation of telomere length. *Science* **1990**, *250*, 549–553. [[CrossRef](#)]
91. Wotton, D.; Shore, D. A novel Rap1p-interacting factor, Rif2p, cooperates with Rif1p to regulate telomere length in *Saccharomyces cerevisiae*. *Genes Dev.* **1997**, *11*, 748–760. [[CrossRef](#)] [[PubMed](#)]
92. Marcand, S.; Pardo, B.; Gratias, A.; Cahun, S.; Callebaut, I. Multiple pathways inhibit NHEJ at telomeres. *Genes Dev.* **2008**, *22*, 1153–1158. [[CrossRef](#)] [[PubMed](#)]
93. Ritchie, K.B.; Petes, T.D. The Mre11p/Rad50p/Xrs2p complex and the Tel1p function in a single pathway for telomere maintenance in yeast. *Genetics* **2000**, *155*, 475–479. [[CrossRef](#)] [[PubMed](#)]
94. Hirano, Y.; Fukunaga, K.; Sugimoto, K. Rif1 and Rif2 inhibit localization of Tel1 to DNA ends. *Mol. Cell* **2009**, *33*, 312–322. [[CrossRef](#)] [[PubMed](#)]
95. Anbalagan, S.; Bonetti, D.; Lucchini, G.; Longhese, M.P. Rif1 supports the function of the CST complex in yeast telomere capping. *PLoS Genet.* **2011**, *7*, e1002024. [[CrossRef](#)] [[PubMed](#)]
96. de Lange, T. Shelterin-mediated telomere protection. *Annu. Rev. Genet.* **2018**, *52*, 223–247. [[CrossRef](#)] [[PubMed](#)]
97. Denchi, E.L.; de Lange, T. Protection of telomeres through independent control of ATM and ATR by TRF2 and POT1. *Nature* **2007**, *448*, 1068–1071. [[CrossRef](#)] [[PubMed](#)]
98. van Steensel, B.; Smogorzewska, A.; de Lange, T. TRF2 protects human telomeres from end-to-end fusions. *Cell* **1998**, *92*, 401–413. [[CrossRef](#)]
99. Griffith, J.D.; Comeau, L.; Rosenfield, S.; Stansel, R.M.; Bianchi, A.; Moss, H.; de Lange, T. Mammalian telomeres end in a large duplex loop. *Cell* **1999**, *97*, 503–514. [[CrossRef](#)]
100. Stansel, R.M.; de Lange, T.; Griffith, J.D. T-loop assembly in vitro involves binding of TRF2 near the 3' telomeric overhang. *EMBO J.* **2001**, *20*, 5532–5540. [[CrossRef](#)] [[PubMed](#)]
101. Karlseder, J.; Hoke, K.; Mirzoeva, O.K.; Bakkenist, C.; Kastan, M.B.; Petrini, J.H.; de Lange, T. The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. *PLoS Biol.* **2004**, *2*, E240. [[CrossRef](#)] [[PubMed](#)]
102. Caracciolo, D.; Riillo, C.; Di Martino, M.T.; Tagliaferri, P.; Tassone, P. Alternative non-homologous end-joining: Error-prone DNA repair as cancer's achilles' heel. *Cancers* **2021**, *13*, 1392. [[CrossRef](#)] [[PubMed](#)]

103. Jones, R.E.; Oh, S.; Grimstead, J.W.; Zimbric, J.; Roger, L.; Heppel, N.H.; Ashelford, K.E.; Liddiard, K.; Hendrickson, E.A.; Baird, D.M. Escape from telomere-driven crisis is DNA ligase III dependent. *Cell Rep.* **2014**, *8*, 1063–1076. [[CrossRef](#)] [[PubMed](#)]
104. Sfeir, A.; de Lange, T. Removal of shelterin reveals the telomere end-protection problem. *Science* **2012**, *336*, 593–597. [[CrossRef](#)] [[PubMed](#)]
105. Graham, I.R.; Chambers, A. Use of a selection technique to identify the diversity of binding sites for the yeast RAP1 transcription factor. *Nucleic Acids Res.* **1994**, *22*, 124–130. [[CrossRef](#)] [[PubMed](#)]
106. Konig, P.; Giraldo, R.; Chapman, L.; Rhodes, D. The crystal structure of the DNA-binding domain of yeast RAP1 in complex with telomeric DNA. *Cell* **1996**, *85*, 125–136. [[CrossRef](#)]
107. Taylor, H.O.; O'Reilly, M.; Leslie, A.G.; Rhodes, D. How the multifunctional yeast Rap1p discriminates between DNA target sites: A crystallographic analysis. *J. Mol. Biol.* **2000**, *303*, 693–707. [[CrossRef](#)] [[PubMed](#)]
108. Feldmann, E.A.; Galletto, R. The DNA-binding domain of yeast Rap1 interacts with double-stranded DNA in multiple binding modes. *Biochemistry* **2014**, *53*, 7471–7483. [[CrossRef](#)] [[PubMed](#)]
109. Feldmann, E.A.; De Bona, P.; Galletto, R. The wrapping loop and Rap1 C-terminal (RCT) domain of yeast Rap1 modulate access to different DNA binding modes. *J. Biol. Chem.* **2015**, *290*, 11455–11466. [[CrossRef](#)] [[PubMed](#)]
110. Bonetti, D.; Rinaldi, C.; Vertemara, J.; Notaro, M.; Pizzul, P.; Tisi, R.; Zampella, G.; Longhese, M.P. DNA binding modes influence Rap1 activity in the regulation of telomere length and MRX functions at DNA ends. *Nucleic Acids Res.* **2020**, *48*, 2424–2441. [[CrossRef](#)] [[PubMed](#)]
111. Hailemariam, S.; De Bona, P.; Galletto, R.; Hohl, M.; Petrini, J.H.; Burgers, P.M. The telomere-binding protein Rif2 and ATP-bound Rad50 have opposing roles in the activation of yeast Tel1ATM kinase. *J. Biol. Chem.* **2019**, *294*, 18846–18852. [[CrossRef](#)] [[PubMed](#)]
112. Liu, Y.; Sung, S.; Kim, Y.; Li, F.; Gwon, G.; Jo, A.; Kim, A.K.; Kim, T.; Song, O.K.; Lee, S.E.; et al. ATP-dependent DNA binding, unwinding, and resection by the Mre11/Rad50 complex. *EMBO J.* **2016**, *35*, 743–758. [[CrossRef](#)] [[PubMed](#)]
113. Seifert, F.U.; Lammens, K.; Stoehr, G.; Kessler, B.; Hopfner, K.P. Structural mechanism of ATP-dependent DNA binding and DNA end bridging by eukaryotic Rad50. *EMBO J.* **2016**, *35*, 759–772. [[CrossRef](#)] [[PubMed](#)]
114. Hailemariam, S.; Kumar, S.; and Burgers, P.M. Activation of Tel1ATM kinase requires Rad50 ATPase and long nucleosome-free DNA but no DNA ends. *J. Biol. Chem.* **2019**, *294*, 10120–10130. [[CrossRef](#)] [[PubMed](#)]
115. Khayat, F.; Cannavo, E.; Alshmary, M.; Foster, W.R.; Chahwan, C.; Maddalena, M.; Smith, C.; Oliver, A.W.; Watson, A.T.; Carr, A.M.; et al. Inhibition of MRN activity by a telomere protein motif. *Nat. Commun.* **2021**, *12*, 3856. [[CrossRef](#)] [[PubMed](#)]
116. Marsella, A.; Gobbin, E.; Cassani, C.; Tisi, R.; Cannavo, E.; Reginato, G.; Cejka, P.; Longhese, M.P. Sae2 and Rif2 regulate MRX endonuclease activity at DNA double-strand breaks in opposite manners. *Cell Rep.* **2021**, *34*, 108906. [[CrossRef](#)] [[PubMed](#)]
117. Roisé-Hamelin, F.; Pobiega, S.; Jézéquel, K.; Miron, S.; Dépagne, J.; Veaute, X.; Busso, D.; Du, M.L.; Callebaut, I.; Charbonnier, J.B.; et al. Mechanism of MRX inhibition by Rif2 at telomeres. *Nat. Commun.* **2021**, *12*, 2763. [[CrossRef](#)] [[PubMed](#)]
118. Cannavo, E.; Johnson, D.; Andres, S.N.; Kissling, V.M.; Reinert, J.K.; Garcia, V.; Erie, D.A.; Hess, D.; Thomä, N.H.; Enchev, R.I.; et al. Regulatory control of DNA end resection by Sae2 phosphorylation. *Nat. Commun.* **2018**, *9*, 4016. [[CrossRef](#)] [[PubMed](#)]
119. Kaizer, H.; Connelly, C.J.; Bettridge, K.; Viggiani, C.; Greider, C.W. Regulation of telomere length requires a conserved N-terminal domain of Rif2 in *Saccharomyces cerevisiae*. *Genetics* **2015**, *201*, 573–586. [[CrossRef](#)] [[PubMed](#)]
120. Hayflick, L.; Moorhead, P.S. The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **1961**, *25*, 585–621. [[CrossRef](#)]
121. Watson, J. Origin of concatemeric T7 DNA. *Nat. New Biol.* **1972**, *239*, 197–201. [[CrossRef](#)] [[PubMed](#)]
122. Olovnikov, A.M. 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. [[CrossRef](#)]
123. Harley, C.B.; Futcher, A.B.; Greider, C.W. Telomeres shorten during ageing of human fibroblasts. *Nature* **1990**, *345*, 458–460. [[CrossRef](#)] [[PubMed](#)]
124. Bodnar, A.G.; Ouellette, M.; Frolkis, M.; Holt, S.E.; Chiu, C.P.; Morin, G.B.; Harley, C.B.; Shay, J.W.; Lichtsteiner, S.; Wright, W.E. Extension of life-span by introduction of telomerase into normal human cells. *Science* **1998**, *279*, 349–352. [[CrossRef](#)] [[PubMed](#)]
125. Hackett, J.A.; Feldser, D.M.; Greider, C.W. Telomere dysfunction increases mutation rate and genomic instability. *Cell* **2001**, *106*, 275–286. [[CrossRef](#)]
126. Enomoto, S.; Glowczewski, L.; Berman, J. MEC3, MEC1, and DDC2 are essential components of a telomere checkpoint pathway required for cell cycle arrest during senescence in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **2002**, *13*, 2626–2638. [[CrossRef](#)] [[PubMed](#)]
127. Ijpm, A.S.; Greider, C.W. Short telomeres induce a DNA damage response in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **2003**, *14*, 987–1001. [[CrossRef](#)] [[PubMed](#)]
128. d'Adda di Fagagna, F.; Reaper, P.M.; Clay-Farrace, L.; Fiegler, H.; Carr, P.; Von Zglinicki, T.; Saretzki, G.; Carter, N.P.; Jackson, S.P. A DNA damage checkpoint response in telomere-initiated senescence. *Nature* **2003**, *426*, 194–198. [[CrossRef](#)] [[PubMed](#)]
129. Herbig, U.; Jobling, W.A.; Chen, B.P.; Chen, D.J.; Sedivy, J.M. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol. Cell* **2004**, *14*, 501–513. [[CrossRef](#)]
130. Karlseder, J.; Smogorzewska, A.; de Lange, T. Senescence induced by altered telomere state, not telomere loss. *Science* **2002**, *295*, 2446–2449. [[CrossRef](#)] [[PubMed](#)]
131. Cesare, A.J.; Karlseder, J. A three-state model of telomere control over human proliferative boundaries. *Curr. Opin. Cell Biol.* **2012**, *24*, 731–738. [[CrossRef](#)] [[PubMed](#)]

132. Capper, R.; Britt-Compton, B.; Tankimanova, M.; Rowson, J.; Letsolo, B.; Man, S.; Haughton, M.; Baird, D.M. The nature of telomere fusion and a definition of the critical telomere length in human cells. *Genes Dev.* **2007**, *21*, 2495–2508. [[CrossRef](#)] [[PubMed](#)]
133. Letsolo, B.T.; Rowson, J.; Baird, D.M. Fusion of short telomeres in human cells is characterized by extensive deletion and microhomology, and can result in complex rearrangements. *Nucleic Acids Res.* **2010**, *38*, 1841–1852. [[CrossRef](#)] [[PubMed](#)]
134. Di Micco, R.; Krizhanovsky, V.; Baker, D.; d’Adda di Fagagna, F. Cellular senescence in ageing: From mechanisms to therapeutic opportunities. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 75–95. [[CrossRef](#)] [[PubMed](#)]
135. Abdallah, P.; Luciano, P.; Runge, K.W.; Lisby, M.; Géli, V.; Gilson, E.; Teixeira, M.T. A two-step model for senescence triggered by a single critically short telomere. *Nat. Cell Biol.* **2009**, *11*, 988–993. [[CrossRef](#)] [[PubMed](#)]
136. Khadaroo, B.; Teixeira, M.T.; Luciano, P.; Eckert-Boulet, N.; Germann, S.M.; Simon, M.N.; Gallina, I.; Abdallah, P.; Gilson, E.; Géli, V.; et al. The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. *Nat. Cell Biol.* **2009**, *11*, 980–987. [[CrossRef](#)] [[PubMed](#)]
137. Hemann, M.T.; Strong, M.A.; Hao, L.Y.; Greider, C.W. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* **2001**, *107*, 67–77. [[CrossRef](#)]
138. Fallet, E.; Jolivet, P.; Soudet, J.; Lisby, M.; Gilson, E.; Teixeira, M.T. Length-dependent processing of telomeres in the absence of telomerase. *Nucleic Acids Res.* **2014**, *42*, 3648–3665. [[CrossRef](#)] [[PubMed](#)]
139. Ballew, B.J.; Lundblad, V. Multiple genetic pathways regulate replicative senescence in telomerase-deficient yeast. *Aging Cell* **2013**, *12*, 719–727. [[CrossRef](#)] [[PubMed](#)]
140. Chang, M.; Rothstein, R. Rif1/2 and Tel1 function in separate pathways during replicative senescence. *Cell Cycle* **2011**, *10*, 3798–3799. [[CrossRef](#)] [[PubMed](#)]
141. Mantiero, D.; Clerici, M.; Lucchini, G.; Longhese, M.P. Dual role for *Saccharomyces cerevisiae* Tel1 in the checkpoint response to double-strand breaks. *EMBO Rep.* **2007**, *8*, 380–387. [[CrossRef](#)]
142. Baldo, V.; Testoni, V.; Lucchini, G.; Longhese, M.P. Dominant *TEL1-hy* mutations compensate for Mec1 lack of functions in the DNA damage response. *Mol. Cell Biol.* **2008**, *28*, 358–375. [[CrossRef](#)] [[PubMed](#)]
143. Menin, L.; Colombo, C.V.; Maestrini, G.; Longhese, M.P.; Clerici, M. Tel1/ATM signaling to the checkpoint contributes to replicative senescence in the absence of telomerase. *Genetics* **2019**, *213*, 411–429. [[CrossRef](#)] [[PubMed](#)]
144. Artandi, S.E.; Chang, S.; Lee, S.L.; Alson, S.; Gottlieb, G.J.; Chin, L.; DePinho, R.A. Telomere dysfunction promotes non-reciprocal translocations and epithelial cancers in mice. *Nature* **2000**, *406*, 641–645. [[CrossRef](#)]
145. Toczyski, D.P.; Galgoczy, D.J.; Hartwell, L.H. CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. *Cell* **1997**, *90*, 1097–1106. [[CrossRef](#)]
146. Lee, S.E.; Moore, J.K.; Holmes, A.; Umez, K.; Kolodner, R.D.; Haber, J.E. *Saccharomyces* Ku70, Mre11/Rad50 and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **1998**, *94*, 399–409. [[CrossRef](#)]
147. Pellicioli, A.; Lee, S.E.; Lucca, C.; Foiani, M.; Haber, J.E. Regulation of *Saccharomyces* Rad53 checkpoint kinase during adaptation from DNA damage-induced G2/M arrest. *Mol. Cell* **2001**, *7*, 293–300. [[CrossRef](#)]
148. Leroy, C.; Lee, S.E.; Vaze, M.B.; Ochsenein, F.; Guerois, R.; Haber, J.E.; Marsolier-Kergoat, M.C. PP2C phosphatases Ptc2 and Ptc3 are required for DNA checkpoint inactivation after a double-strand break. *Mol. Cell* **2003**, *11*, 827–835. [[CrossRef](#)]
149. Lee, S.E.; Pellicioli, A.; Malkova, A.; Foiani, M.; Haber, J.E. The *Saccharomyces* recombination protein Tid1p is required for adaptation from G2/M arrest induced by a double-strand break. *Curr. Biol.* **2001**, *11*, 1053–1057. [[CrossRef](#)]
150. Galgoczy, D.J.; Toczyski, D.P. Checkpoint adaptation precedes spontaneous and damage-induced genomic instability in yeast. *Mol. Cell Biol.* **2001**, *21*, 1710–1718. [[CrossRef](#)] [[PubMed](#)]
151. Kaye, J.A.; Melo, J.A.; Cheung, S.K.; Vaze, M.B.; Haber, J.E.; Toczyski, D.P. DNA breaks promote genomic instability by impeding proper chromosome segregation. *Curr. Biol.* **2004**, *14*, 2096–2106. [[CrossRef](#)] [[PubMed](#)]
152. Yoo, H.Y.; Kumagai, A.; Shevchenko, A.; Shevchenko, A.; Dunphy, W.G. Adaptation of a DNA replication checkpoint response depends upon inactivation of Claspin by the Polo-like kinase. *Cell* **2004**, *117*, 575–588. [[CrossRef](#)]
153. Syljuåsen, R.G.; Jensen, S.; Bartek, J.; Lukas, J. Adaptation to the ionizing radiation-induced G2 checkpoint occurs in human cells and depends on checkpoint kinase 1 and Polo-like kinase 1 kinases. *Cancer Res.* **2006**, *66*, 10253–10257. [[CrossRef](#)]
154. Mersaoui, S.Y.; Gravel, S.; Karpov, V.; Wellinger, R.J. DNA damage checkpoint adaptation genes are required for division of cells harbouring eroded telomeres. *Microb. Cell* **2015**, *2*, 394–405. [[CrossRef](#)] [[PubMed](#)]
155. Coutelier, H.; Xu, Z.; Morisse, M.C.; Lhuillier-Akakpo, M.; Pelet, S.; Charvin, G.; Dubrana, K.; Teixeira, M.T. Adaptation to DNA damage checkpoint in senescent telomerase-negative cells promotes genome instability. *Genes Dev.* **2018**, *32*, 1499–1513. [[CrossRef](#)] [[PubMed](#)]
156. Beyer, T.; Weinert, T. Ontogeny of unstable chromosomes generated by telomere error in budding yeast. *PLoS Genet.* **2016**, *12*, e1006345. [[CrossRef](#)] [[PubMed](#)]
157. Hahn, W.C.; Counter, C.M.; Lundberg, A.S.; Beijersbergen, R.L.; Brooks, M.W.; Weinberg, R.A. Creation of human tumour cells with defined genetic elements. *Nature* **1999**, *400*, 464–468. [[CrossRef](#)] [[PubMed](#)]
158. Lundblad, V.; Blackburn, E.H. An alternative pathway for yeast telomere maintenance rescues est1- senescence. *Cell* **1993**, *73*, 347–360. [[CrossRef](#)]
159. Teng, S.C.; Zakian, V.A. Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **1999**, *19*, 8083–8093. [[CrossRef](#)]

160. Chen, Q.; Ijpm, A.; Greider, C.W. Two survivor pathways that allow growth in the absence of telomerase are generated by distinct telomere recombination events. *Mol. Cell Biol.* **2001**, *21*, 1819–1827. [[CrossRef](#)]
161. Maringele, L.; Lydall, D. Telomerase- and recombination-independent immortalization of budding yeast. *Genes Dev.* **2004**, *18*, 2663–2675. [[CrossRef](#)] [[PubMed](#)]
162. Grandin, N.; Charbonneau, M. Telomerase- and Rad52-independent immortalization of budding yeast by an inherited-long-telomere pathway of telomeric repeat amplification. *Mol. Cell Biol.* **2009**, *29*, 965–985. [[CrossRef](#)]
163. Lebel, C.; Rosonina, E.; Sealey, D.C.; Pryde, F.; Lydall, D.; Maringele, L.; Harrington, L.A. Telomere maintenance and survival in *Saccharomyces cerevisiae* in the absence of telomerase and RAD52. *Genetics* **2009**, *182*, 671–684. [[CrossRef](#)]
164. Larrivée, M.; Wellinger, R.J. Telomerase- and capping-independent yeast survivors with alternate telomere states. *Nat. Cell Biol.* **2006**, *8*, 741–747. [[CrossRef](#)] [[PubMed](#)]
165. Wellinger, R.J.; Wolf, A.J.; Zakian, V.A. *Saccharomyces* telomeres acquire single-strand TG₁₋₃ tails late in S phase. *Cell* **1993**, *72*, 51–60. [[CrossRef](#)]
166. McEachern, M.J.; Blackburn, E.H. A conserved sequence motif within the exceptionally diverse telomeric sequences of budding yeasts. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 3453–3457. [[CrossRef](#)] [[PubMed](#)]
167. Le, S.; Moore, J.K.; Haber, J.E.; Greider, C.W. RAD50 and RAD51 define two pathways that collaborate to maintain telomeres in the absence of telomerase. *Genetics* **1999**, *152*, 143–152. [[CrossRef](#)]
168. Teng, S.C.; Chang, J.; McCowan, B.; Zakian, V.A. Telomerase-independent lengthening of yeast telomeres occurs by an abrupt Rad50p-dependent, Rif-inhibited recombinational process. *Mol. Cell* **2000**, *6*, 947–952. [[CrossRef](#)]
169. Johnson, F.B.; Marciniak, R.A.; McVey, M.; Stewart, S.A.; Hahn, W.C.; Guarente, L. The *Saccharomyces cerevisiae* WRN homolog Sgs1p participates in telomere maintenance in cells lacking telomerase. *EMBO J.* **2001**, *20*, 905–913. [[CrossRef](#)] [[PubMed](#)]
170. Huang, P.; Pryde, F.E.; Lester, D.; Maddison, R.L.; Borts, R.H.; Hickson, I.D.; Louis, E.J. SGS1 is required for telomere elongation in the absence of telomerase. *Curr. Biol.* **2001**, *11*, 125–129. [[CrossRef](#)]
171. Lydeard, J.R.; Jain, S.; Yamaguchi, M.; Haber, J.E. Break-induced replication and telomerase-independent telomere maintenance require Pol32. *Nature* **2007**, *448*, 820–823. [[CrossRef](#)]
172. Kockler, Z.W.; Comeron, J.M.; Malkova, A. A unified alternative telomere-lengthening pathway in yeast survivor cells. *Mol. Cell* **2021**, *81*, 1816–1829. [[CrossRef](#)]
173. Makovets, S.; Williams, T.L.; Blackburn, E.H. The telotype defines the telomere state in *Saccharomyces cerevisiae* and is inherited as a dominant non-Mendelian characteristic in cells lacking telomerase. *Genetics* **2008**, *178*, 245–257. [[CrossRef](#)]
174. Becerra, S.C.; Thambugala, H.T.; Erickson, A.R.; Lee, C.K.; Lewis, L.K. Reversibility of replicative senescence in *Saccharomyces cerevisiae*: Effect of homologous recombination and cell cycle checkpoints. *DNA Repair* **2012**, *11*, 35–45. [[CrossRef](#)]
175. Nakamura, T.M.; Cooper, J.P.; Cech, T.R. Two modes of survival of fission yeast without telomerase. *Science* **1998**, *282*, 493–496. [[CrossRef](#)] [[PubMed](#)]
176. Wang, X.; Baumann, P. Chromosome fusions following telomere loss are mediated by single-strand annealing. *Mol. Cell* **2008**, *31*, 463–473. [[CrossRef](#)] [[PubMed](#)]
177. Almeida, H.; Godinho Ferreira, M. Spontaneous telomere to telomere fusions occur in unperturbed fission yeast cells. *Nucleic Acids Res.* **2013**, *41*, 3056–3067. [[CrossRef](#)] [[PubMed](#)]
178. Jain, D.; Hebden, A.K.; Nakamura, T.M.; Miller, K.M.; Cooper, J.P. HAATI survivors replace canonical telomeres with blocks of generic heterochromatin. *Nature* **2010**, *467*, 223–227. [[CrossRef](#)]
179. Bryan, T.M.; Englezou, A.; Dalla-Pozza, L.; Dunham, M.A.; Reddel, R.R. Evidence for an alternative mechanism for maintaining telomere length in human tumors and tumor-derived cell lines. *Nat. Med.* **1997**, *3*, 1271–1274. [[CrossRef](#)] [[PubMed](#)]
180. Gao, J.; Pickett, H.A. Targeting telomeres: Advances in telomere maintenance mechanism-specific cancer therapies. *Nat. Rev. Cancer* **2022**, *22*, 515–532. [[CrossRef](#)]
181. Dunham, M.A.; Neumann, A.A.; Fasching, C.L.; Reddel, R.R. Telomere maintenance by recombination in human cells. *Nat. Genet.* **2000**, *26*, 447–450. [[CrossRef](#)] [[PubMed](#)]
182. Muntoni, A.; Neumann, A.A.; Hills, M.; Reddel, R.R. Telomere elongation involves intra-molecular DNA replication in cells utilizing alternative lengthening of telomeres. *Hum. Mol. Genet.* **2009**, *18*, 1017–1027. [[CrossRef](#)] [[PubMed](#)]
183. Neumann, A.A.; Watson, C.M.; Noble, J.R.; Pickett, H.A.; Tam, P.P.; Reddel, R.R. Alternative lengthening of telomeres in normal mammalian somatic cells. *Genes Dev.* **2013**, *27*, 18–23. [[CrossRef](#)]
184. Sfeir, A.; Kosiyatrakul, S.T.; Hockemeyer, D.; MacRae, S.L.; Karlseder, J.; Schildkraut, C.L.; de Lange, T. Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. *Cell* **2009**, *138*, 90–103. [[CrossRef](#)] [[PubMed](#)]
185. Grandin, N.; Charbonneau, M. Mrc1, a non-essential DNA replication protein, is required for telomere end protection following loss of capping by Cdc13, Yku or telomerase. *Mol. Genet. Genomics* **2007**, *277*, 685–699. [[CrossRef](#)]
186. Grandin, N.; Bailly, A.; Charbonneau, M. Activation of Mrc1, a mediator of the replication checkpoint, by telomere erosion. *Biol. Cell* **2005**, *97*, 799–814. [[CrossRef](#)]
187. Lee, J.Y.; Kozak, M.; Martin, J.D.; Pennock, E.; Johnson, F.B. Evidence that a RecQ helicase slows senescence by resolving recombining telomeres. *PLoS Biol.* **2007**, *5*, e160. [[CrossRef](#)]
188. Matmati, S.; Lambert, S.; Géli, V.; Coulon, S. Telomerase repairs collapsed replication forks at telomeres. *Cell Rep.* **2020**, *30*, 3312–3322. [[CrossRef](#)]

189. Pickett, H.A.; Reddel, R.R. Molecular mechanisms of activity and derepression of alternative lengthening of telomeres. *Nat. Struct. Mol. Biol.* **2015**, *22*, 875–880. [[CrossRef](#)]
190. Sobinoff, A.P.; Pickett, H.A. Alternative lengthening of telomeres: DNA repair pathways converge. *Trends Genet.* **2017**, *33*, 921–932. [[CrossRef](#)]
191. Kim, J.; Sun, C.; Tran, A.D.; Chin, P.J.; Ruiz, P.D.; Wang, K.; Gibbons, R.J.; Gamble, M.J.; Liu, Y.; Oberdoerffer, P. The macroH2A1.2 histone variant links ATRX loss to alternative telomere lengthening. *Nat. Struct. Mol. Biol.* **2019**, *26*, 213–219. [[CrossRef](#)] [[PubMed](#)]
192. Li, F.; Deng, Z.; Zhang, L.; Wu, C.; Jin, Y.; Hwang, I.; Vladimirova, O.; Xu, L.; Yang, L.; Lu, B.; et al. ATRX loss induces telomere dysfunction and necessitates induction of alternative lengthening of telomeres during human cell immortalization. *EMBO J.* **2019**, *38*, e96659. [[CrossRef](#)] [[PubMed](#)]
193. Clynes, D.; Jelinska, C.; Xella, B.; Ayyub, H.; Scott, C.; Mitson, M.; Taylor, S.; Higgs, D.R.; Gibbons, R.J. Suppression of the alternative lengthening of telomere pathway by the chromatin remodelling factor ATRX. *Nat. Commun.* **2015**, *6*, 7538. [[CrossRef](#)] [[PubMed](#)]
194. Nguyen, D.T.; Voon, H.P.J.; Xella, B.; Scott, C.; Clynes, D.; Babbs, C.; Ayyub, H.; Kerry, J.; Sharpe, J.A.; Sloane-Stanley, J.A.; et al. The chromatin remodelling factor ATRX suppresses R-loops in transcribed telomeric repeats. *EMBO Rep.* **2017**, *18*, 914–928. [[CrossRef](#)]
195. O’Sullivan, R.J.; Almouzni, G. Assembly of telomeric chromatin to create ALternative endings. *Trends Cell Biol.* **2014**, *24*, 675–685. [[CrossRef](#)]
196. Maicher, A.; Kastner, L.; Dees, M.; Luke, B. Deregulated telomere transcription causes replication-dependent telomere shortening and promotes cellular senescence. *Nucleic Acids Res.* **2012**, *40*, 5966–6649. [[CrossRef](#)]
197. Cusanelli, E.; Romero, C.A.; Chartrand, P. Telomeric noncoding RNA TERRA is induced by telomere shortening to nucleate telomerase molecules at short telomeres. *Mol. Cell* **2013**, *51*, 780–791. [[CrossRef](#)]
198. Moravec, M.; Wischniewski, H.; Bah, A.; Hu, Y.; Liu, N.; Lafranchi, L.; King, M.C.; Azzalin, C.M. TERRA promotes telomerase-mediated telomere elongation in *Schizosaccharomyces pombe*. *EMBO Rep.* **2016**, *17*, 999–1012. [[CrossRef](#)]
199. Hu, Y.; Bennett, H.W.; Liu, N.; Moravec, M.; Williams, J.F.; Azzalin, C.M.; King, M.C. RNA-DNA hybrids support recombination-based telomere maintenance in fission yeast. *Genetics* **2019**, *213*, 431–447. [[CrossRef](#)]
200. Balk, B.; Maicher, A.; Dees, M.; Klermund, J.; Luke-Glaser, S.; Bender, K.; Luke, B. Telomeric RNA-DNA hybrids affect telomere-length dynamics and senescence. *Nat. Struct. Mol. Biol.* **2013**, *20*, 1199–1205. [[CrossRef](#)]
201. Yu, T.Y.; Kao, Y.W.; Lin, J.J. Telomeric transcripts stimulate telomere recombination to suppress senescence in cells lacking telomerase. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 3377–3382. [[CrossRef](#)] [[PubMed](#)]
202. Graf, M.; Bonetti, D.; Lockhart, A.; Serhal, K.; Kellner, V.; Maicher, A.; Jolivet, P.; Teixeira, M.T.; Luke, B. Telomere length determines TERRA and R-loop regulation through the cell cycle. *Cell* **2017**, *170*, 72–85. [[CrossRef](#)]
203. Silva, B.; Arora, R.; Bione, S.; Azzalin, C.M. TERRA transcription destabilizes telomere integrity to initiate break-induced replication in human ALT cells. *Nat. Commun.* **2021**, *12*, 1–12. [[CrossRef](#)] [[PubMed](#)]
204. Rudolph, K.L.; Millard, M.; Bosenberg, M.W.; DePinho, R.A. Telomere dysfunction and evolution of intestinal carcinoma in mice and humans. *Nat. Genet.* **2001**, *28*, 155–159. [[CrossRef](#)] [[PubMed](#)]
205. Ding, Z.; Wu, C.J.; Jaskelioff, M.; Ivanova, E.; Kost-Alimova, M.; Protopopov, A.; Chu, G.C.; Wang, G.; Lu, X.; Labrot, E.S.; et al. Telomerase reactivation following telomere dysfunction yields murine prostate tumors with bone metastases. *Cell* **2012**, *148*, 896–907. [[CrossRef](#)]
206. Hu, J.; Hwang, S.S.; Liesa, M.; Gan, B.; Sahin, E.; Jaskelioff, M.; Ding, Z.; Ying, H.; Boutin, A.T.; Zhang, H.; et al. Antitelomerase therapy provokes ALT and mitochondrial adaptive mechanisms in cancer. *Cell* **2012**, *148*, 651–663. [[CrossRef](#)]