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Review

Regulation of homologous recombination at telomeres in budding yeast

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ABSTRACT

Homologous recombination is suppressed at normal length telomere sequences. In contrast, telomere recombination is allowed when telomeres erode in the absence of telomerase activity or as a consequence of nucleolytic degradation or incomplete replication. Here, we review the mechanisms that contribute to regulating mitotic homologous recombination at telomeres and the role of these mechanisms in signalling short telomeres in the budding yeast *Saccharomyces cerevisiae*.

1. Introduction

Telomeres are conserved nucleo-protein complexes that define the physical ends of eukaryotic chromosomes and ensure their stability by facilitating efficient replication and by preventing untimely recognition by the DNA damage machinery. Telomere length is dynamic and primarily determined by the balance between loss of telomere sequences due to the "end-replication problem", i.e. the inability of DNA polymerase to fully replicate the telomere termini, and gain of telomere sequences by telomerase-mediated extension of telomere ends. Telomerase is a reverse transcriptase with an intrinsic RNA template [1] and was first identified in *Tetrahymena* [2]. In *Saccharomyces cerevisiae*, telomerase comprises the RNA template *TLC1* and the catalytic subunit Est2 [3–5]. *TLC1* anneals to the extreme terminus of the telomeres and is used as a template for telomere extension by Est2 [5]. Regulation of telomerase activity in vivo additionally requires Cdc13, Est1 and Est3, although these proteins are not directly involved in the catalytic activity of telomerase [6,7]. Deletion of *EST1* or *EST3* results in shorter telomeres (EST: Ever Shorter Telomeres) and in a replicative senescence phenotype [7,8]. Est1 has been shown to associate with telomerase via *TLC1* [9], while Est3 associates with telomerase via an OB-fold domain [10]. Since telomeres are the physical ends of chromosomes, they resemble DNA double-strand breaks and are potential targets for the DNA repair machinery.

DNA repair is vital for cell survival, because DNA damage continuously arises as a result of exogenous or endogenous DNA damaging agents or as a result of problems during DNA replication. Double-strand breaks (DSBs), in which both DNA strands are broken, are some of the most deleterious lesions. Two pathways can repair this kind of damage: non-homologous end-joining (NHEJ), which may require nucleolytic trimming of the DNA at the break before ligation of the ends [11], and homologous recombination (HR) [12]. While NHEJ is error-prone, HR is generally considered error-free, because genetic information is copied from an intact homologous duplex to restore genetic information disrupted at the DSB. While NHEJ is the predominant DSB repair pathway in human cells, HR is preferred in the budding yeast *S. cerevisiae*. DSBs are recognized and processed by numerous proteins [13]. When a DSB occurs, it is first bound by the yKu70–yKu80 (Ku) and/or the Mre11-Rad50-Xrs2 (MRX) complexes in a competitive fashion [14–16]. The Ku complex inhibits DSB resection and thereby promotes NHEJ [14], while MRX favors resection, which directs repair to the homologous recombination pathway. In addition to the intrinsic nuclease activity of MRX, the Sae2, Sgs1, Dna2 and Exo1 enzymes also contribute to resection of DSBs [17]. The MRX complex recruits the Tel1 kinase [18] to signal the initial checkpoint response to a DSB [19,20]. Further, MRX plays a structural role in tethering the two ends of a DSB [21,22]. In budding yeast, the resection of a DSB results in the loss of MRX and Tel1 association [23]. However, the resulting single-stranded DNA (ssDNA) is bound by replication protein A (RPA), which recruits the Mec1-Ddc2 (ATR-ATRIP) and the Ddc1-Mec3-Rad17 (9-1-1) complexes to maintain DNA damage checkpoint signalling. RPA also recruits

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Rad52 to initiate homologous recombination. Rad52 catalyzes displacement of RPA and loads the Rad51 recombinase onto the ssDNA, thus mediating the formation of a Rad51 filament. Rad51 is responsible for homology search in order to find a homologous donor sequence, from which the genetic information can be copied after strand invasion has occurred.

Homologous recombination is regulated at several levels to prevent untimely and deleterious recombination. For example, homologous recombination is restricted to the S/G2 phase of the cell cycle by permitting extensive DSB resection only at this phase [24,25]. Further, recombination at the ribosomal gene locus (rDNA) is suppressed by exclusion of Rad52, Rad59, Rad51, and Rad55 from the nucleolus and in part by sumoylation of Rad52 [26]. Several lines of evidence also suggest that homologous recombination is controlled by modification of chromatin structure [27–30].

To prevent the telomere ends of chromosomes from being recognized and processed as DSBs, telomeres are capped by specific proteins including Rap1, and the Sir2-Sir3-Sir4 and Cdc13-Stn1-Ten1 complexes, which prevent the DNA damage response from being activated at telomeres. This is necessary to prevent end-to-end fusion of chromosomes [31].

In the absence of telomerase, telomeres become shorter with each replication cycle. In budding yeast, telomeres erode on average 3–5 bp per mitotic cell cycle, whereas the rate of erosion is 50–100 bp per cell cycle in human cells [3,6,32,33]. Telomere erosion is linked to aging, and it is possible to estimate the age of individuals based on the average length of their telomeres [34,35]. Indeed, transgenic mice with constitutively short telomeres and wild-type telomerase show age-associated degenerative symptoms [36]. Although telomerase is inactive in most mammalian somatic cells, it is active in germ cells. Importantly, telomerase activity is up-regulated in many cancer cells [37]. In *S. cerevisiae*, telomerase is active in wild-type cells. However, telomerase-negative cells continue to divide for approximately 50 generations until they enter a permanent cell cycle arrest, also termed replicative senescence, with critically short telomeres [6].

Nevertheless, a small fraction of telomerase-negative cells survive replicative senescence by alternative telomere lengthening mechanisms. These cells are referred to as survivors. In budding yeast, the predominant mechanism for survivor formation is *RAD52*-dependent recombinational telomere elongation (RTE) [38], which bears resemblance to alternative lengthening of telomeres (ALT) in mammalian cells (reviewed in [39,40]).

2. Telomere homeostasis

Telomeres are composed of GC-rich tandem repeats of a degenerate TG_{1-3} sequence with a 12–14 nt single-stranded 3′ overhang termed the G-tail [41]. A variable number of subtelomeric X and Y′ sequences are found immediately adjacent to the telomeric TG_{1-3} repeats. The length of the tandem TG_{1-3} repeats is maintained in the range of 250–350 bp. Telomere length homeostasis is a dynamic process that is regulated by switching from a state in which telomeres are accessible to telomerase to a state where they are not. As a consequence, telomerase acts preferentially on the shortest telomeres [42].

In *S. cerevisiae*, the Rap1 protein binds the double-stranded telomeric DNA, while Cdc13 binds the single-stranded G-tail [43]. Rap1 is a *cis*-acting regulator that negatively affects telomere elongation by inhibiting resection and telomerase recruitment [44–46]. The longer the double-stranded telomeric DNA, the greater the amount of Rapl bound to it, resulting in inhibition of telomerase activity [33,46].

Cdc13 is a telomere-binding protein that affects telomere length homeostasis in a dual manner [5,47]. *cdc13* mutants were originally found to display stretches of single-stranded DNA at telomere-proximal regions [47]. Cdc13 associates with Ten1 and Stnl [48], which are also able to bind telomeric DNA. The Cdc13-Stn1-Ten1 (CST) complex has been suggested to act as a telomere-specific RPA-like complex [49]. Recent crystallography data support this notion [50]. Binding of Cdc13 to single-stranded telomeric sequences blocks access of the catalytic site of telomerase to the G-tail [51]. Genetic data indicate that the interaction between Stnl and Cdc13 is required for negatively regulating telomerase activity [52]. Ten1 was found to enhance binding of Cdc13 to telomeric DNA, thereby also enhancing telomerase inhibition [53]. Besides this role as a negative regulator, Cdc13 is required for telomerase recruitment and G strand synthesis [43,54]. Thus Cdc13 can act as both a negative and a positive regulator of telomere length homeostasis, depending on whether it is bound to telomerase (positive regulation) or to Stn1 (negative regulation) [54].

3. Telomere capping prevents the DNA repair pathways at chromosome ends

Besides regulating telomere length and telomerase activity, the telomere capping proteins also play a crucial role in protecting telomeres from the DNA repair machinery. The importance of telomere capping in this respect is illustrated by the observation that a conditional mutant of *rap1* results in more frequent chromosome fusions. Since loss of the yKu proteins, Mre11 and Lig4 suppresses this phenotype, chromosome fusions seem to occur by NHEJ [31]. Recently, it has been shown that Rif2, Sir4 and the central domain of Rap1 inhibit NHEJ via several independent pathways [55]. Interestingly, the inhibition of NHEJ by Rap1 and Rif2 does not require the presence of telomeric DNA, since ectopic tethering of the C terminus of Rap1 near a DSB also leads to inhibition of NHEJ. The authors argue that Rif2 thus might act through inhibition of the MRX complex [55], which is consistent with the recent finding that Rif2 competes with Tel1 for binding to the C terminus of Xrs2 [56]. Thus, multiple independent mechanisms are in place to ensure that telomere fusions do not occur under normal circumstances [55,57,58].

Telomere capping by Rap1 also inhibits the HR pathway at telomeres by suppressing recruitment of the Mec1-Ddc2 checkpoint complex and the Rad52, Mre11 and RPA recombination machinery unless telomeres are short [45,59]. For a telomere DSB, this length-dependent regulation of Mre11 and RPA recruitment seems to require Rap1 being bound to the DNA, because the regulation is abolished by mutation of the Rap1-binding sequence [45]. However, some aspects of the cellular response to a telomere DSB may differ from the response to telomeres that shorten gradually, since for example the preference for binding of Mec1 to short telomeric DNA at a DSB is not reflected in a requirement for Mec1 to preferentially extend the shortest telomere during gradual telomere erosion [45,60]. Further, the subtelomeric binding protein Tbf1 also contributes to telomerase-mediated telomere elongation in *tel1*Δ cells [60]. Importantly, the inhibition of recruitment of HR proteins is primarily dependent on the central domain of Rap1 and independent of Ku, Cdc13, the RIF complex and the C-terminal domain of Rap1 [45]. Moreover, uncapping of telomeres as a result of mutation of *RAP1* leads to nucleolytic resection of telomere sequences, which may be the underlying mechanism that destines the telomeres for recombination [45].

4. Senescence and survival pathways

Leonard Hayflick predicted that cells could only undergo a finite number of cell divisions before entering a state of senescence, i.e.

replicative cell cycle arrest [61,62]. This is referred to as the Hayflick limit, and was later proposed to be due to the end-replication problem [63,64]. As stated above, telomerase helps maintaining telomere length, however it is not active in most somatic human cells, because cell differentiation is accompanied by a down-regulation of telomerase gene expression (for review [65]). As a consequence, in normal somatic human cells, telomere length correlates with age [66]. Interestingly, telomerase expression is reactivated in about 85% of human cancers, thus helping the cells to reach a state of immortality and escape senescence. Other mechanisms of telomere lengthening exist that do not involve telomerase: Alternative Lengthening of Telomeres (ALT) is based on recombination processes and is responsible for the escape of senescence in the remaining 15% of cancers (reviewed in [67]).

In *S. cerevisiae*, eroded telomeres, whether caused by mutation of telomerase or other factors that affect telomere length homeostasis, e.g. a conditional *cdc13-2* mutant, may also lead to replicative senescence [43]. However, similar to the ALT pathway in mammalian cells, a small fraction of the cell population survives replicative senescence by extending their telomeres using homologous recombination also referred to as recombinational telomere elongation (RTE). These survivors appear at a frequency of 10^{-4} or less and require *RAD52* [38,68]. Two survivor types exist (reviewed in [40]). Both survival pathways involve break-induced replication like extension of telomere sequences [69,70]. Type I survivors extend their telomeres by amplifying the subtelomeric Y′ elements. Besides *RAD52*, type I survivors require *RAD51*, *RAD54*, *RAD55* and *RAD57*, suggesting that they have formed by Rad51-catalyzed strand invasion (Fig. 1). Telomere length in type I survivors is usually below that of wild-type cells, which may explain their slow growth phenotype. As a consequence, type I survivors are not stable in the long term and because type II survivors grow faster than type I survivors, type II survivors eventually take over in a mixed culture. Type I survivors can convert to type II, but the reverse has not been observed [71]. Type II survivors display sudden elongation of their telomeres by amplification of TG_{1-3} repeats to more than 10 kb. Type II survivor formation is also dependent on *RAD52* but independent on *RAD51*. Further, type II survivor formation requires the helicase Sgs1 (a BLM homologue), the MRX complex and Rad59 [38,72,73], indicating that these survivors are formed by a process that involves strand-displacement (Sgs1), and nucleolytic incision or resection (Sgs1 and MRX) followed by single-strand annealing (Rad59). The checkpoint proteins Tel1 and Mec1 also mediate type II survivor formation [74]. The observation that extrachromosomal telomeric TG_{1-3} and Y′ circles are present in telomerase-deficient cells led to the suggestion that a roll-and-spread mechanism be responsible for the formation of type II survivors [75,76]. However, it is still unclear how Rad51-independent rolling-circle replication of extrachromosomal circles may be established. A possible model for type II survivor formation is presented in Fig. 1. In this model, the helicase activity of Sgs1 unwinds the TG_{1-3} repeats, leading to the appearance of single-stranded DNA. Part of this single-stranded DNA might be excised, resulting in the formation of a DNA circle, which can then anneal to the protruding G-strand and be used as template for elongating the TG_{1-3} repeats.

Using strains in which telomere movements can be visualized by fluorescence microscopy, it has been shown that telomere movements in *est2*Δ or in wild-type cells were similar until the cells enter crisis. At senescence, telomeres travel back and forth across the bud neck in *est2*Δ cells. Post-senescence, the two survivor types can be distinguished by telomere movement: telomeres of type I survivors continue to move back and forth through the bud neck, while telomere movements in type II survivors regain a mobility identical to that of wild-type telomeres [18]. The back-and-forth movements are similar to those observed for a dicentric chromosome during mitosis at the transition to anaphase [77], suggesting that in type I survivors sister chromatids have fused or are linked by unreplicated regions, while the TG_{1-3} extension of telomeres in type II survivors provides wild-type functionality during chromosome replication and propagation.

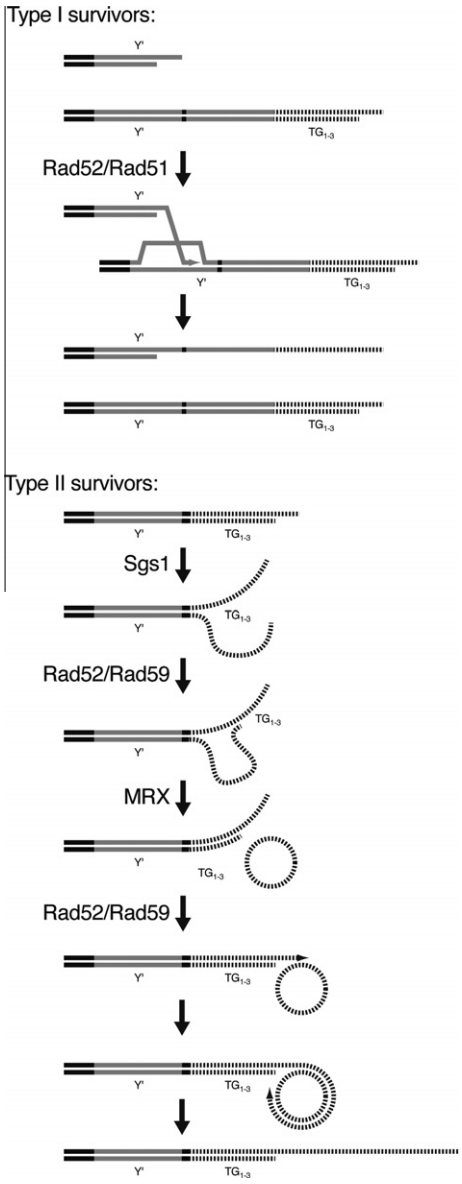

Fig. 1. Recombinational telomere elongation. In the absence of telomerase, rare survivors emerge that maintain their telomeres by homologous recombination in a *RAD52*-dependent manner. Type I survivors extend their telomeres by amplifying the subtelomeric Y′ elements. Besides *RAD52*, type I survivors require *RAD51*, *RAD54*, *RAD55* and *RAD57*, indicating that they have formed by Rad51-catalyzed strand invasion. Type II survivors exhibit sudden elongation of their telomeres by amplification of TG_{1-3} repeats to more than 10 kb. Type II survivor formation is independent on *RAD51*, but requires the 3′–5′ helicase Sgs1, the MRX complex and Rad59. It was further observed that extrachromosomal telomeric TG_{1-3} and Y′ circles were present in telomerase-deficient cells, which led to the suggestion that a roll-and-spread mechanism could be responsible for the formation of type II survivors [75,76]. Several scenarios can be envisioned for the formation of type II survivors. First, Sgs1 could unwind telomeres, which could subsequently reanneal by the aid of Rad59. Due to the repetitiveness of the TG_{1-3} tail, the telomeric repeats may misalign during annealing leading to a loop, which could be excised by the nuclease activity of MRX or other nucleases, leading to formation of a single-stranded loop. This loop could subsequently serve as a template for rolling-circle replication to allow the spreading of the TG_{1-3} repeats to other short telomeres.

5. DNA damage response at eroded telomeres

5.1. Signalling of telomere erosion

Eroded telomeres elicit a DNA damage response which results in Rad53, Rad9, Mrc1, Chk1 and Dun1 phosphorylation, cell cycle arrest and *RNR3* transcriptional induction [78,79]. The DNA damage response is accompanied by enrichment of checkpoint and repair proteins at telomeres in both yeast and mammalian cells [59,80–82]. However, initially eroding telomeres in telomerase-negative cells cause only a transient cell cycle arrest. As a consequence, cells alternate between transient checkpoint arrest and adaptation, a state that is referred to as pre-senescence. Both major DNA damage checkpoint kinases, Mec1 (ATR) and Tel1 (ATM) contribute to the DNA damage response to eroded telomeres [78,83–85].

During pre-senescence, the MRX complex associates with eroded telomeres throughout the cell cycle as evidenced by Mre11 focus formation [59]. In contrast, Ddc2 foci are observed only during S/G2 phase of the cell cycle [59], indicating that although the Mec1-Ddc2 checkpoint kinase is also active during pre-senescence its recruitment to telomeres is cell cycle regulated. These findings are corroborated by chromatin immunoprecipitation of both Tel1 and Mec1 to telomeres [83,86]. Mre11 and Ddc2 focus formation is already induced 10 generations after shutting down *TLC1* transcription, indicating that these proteins are recruited shortly after the telomeres start to shorten. The state of pre-senescence lasts for approximately 50 generations [18,59,83]. However, ultimately the majority of telomeres reach a critical short length of approximately 100 bp, and the majority of cells enter a state of permanent Mec1-dependent checkpoint arrest and eventually die.

Some additional insight into the anatomy of senescence has been gained recently. Using strains in which one telomere was shorter than the others, Abdallah et al. have shown that the presence of a single short telomere is sufficient to accelerate senescence by approximately 15 generations [83]. Surprisingly, the length of the truncated telomere in these telomerase-negative cells remained constant without any evidence of intertelomere recombination even though the population of cells continued to grow, albeit at a reduced rate, suggesting that the subpopulation of cells with the shortest telomere entered a permanent cell cycle arrest, while the remaining cells continued to divide [83]. Further, the authors found that *rad52*Δ and *mms1*Δ strains senesce prematurely without their telomeres being significantly shorter, indicating that the checkpoint signal is stronger in these mutants. Notably, this function of Rad52 during pre-senescence does not involve recombination between the short telomere and the long telomeres. Since the senescence phenotypes of telomerase-negative *rad52*Δ and *mms1*Δ strains were indistinguishable, and because Mms1 is involved in repair of stalled replication forks but not repair of DSBs, the authors conclude that the role of Rad52 in pre-senescence is linked to its function in recombinational restart of stalled replication forks [83]. Perhaps, uncapping of the shortest telomere leads to its resection, which would result in replication fork stalling (Fig. 2). Indeed, a role for Rad52 in preventing resection has been reported for both telomeres and DSBs [40,87]. The observation that Rad52 foci are formed as early as 20 generations after inactivation of *EST2* supports a model in which Rad52 acts already during pre-senescence to rescue stalled replication forks or to prevent excessive resection of telomere termini [59,87], both of which could lead to checkpoint-mediated cell cycle arrest (Fig. 2).

5.2. Localization of eroded telomeres

Yeast telomeres are found close to the nuclear membrane in 6–8 clusters that colocalize with Rap1 as demonstrated by FISH with a probe against the Y′ elements and immunostaining of Rap1 [88]. Recent work indicates that the composition of clusters is dynamic and that interactions between individual telomere pairs are transient and non-specific [89]. Sir4 and Esc1, both involved in silencing, and yKu70 and yKu80 have been shown to tether telomeres to the nuclear envelope [90–92]. Importantly, all telomeres are generally in the vicinity of the nuclear envelope over longer periods of time [90]. A recently identified component of the nuclear envelope in *S. cerevisiae* is the SUN domain protein Mps3 [93,94]. SUN (Sad1-UNC-84) domain proteins are integral membrane proteins found in most eukaryotes. Their conserved C-terminal SUN domain is located in the space between the inner and the outer nuclear membranes, while the acidic N terminus is facing the interior of the nucleus (see [95] for review). Recent studies indicate that Mps3 is involved in anchoring telomeres at the nuclear envelope [92–94]. Pull-down with GST-tagged Mps3 as a bait revealed that Mps3 directly interacts with the telomerase subunit Est1 [93]. Telomere clusters increase in number and telomeres fail to localize at the nuclear envelope in *mps3* mutants [93,94].

During senescence the majority of telomeres remain clustered at multiple Rap1/Ku foci at the nuclear periphery [59]. In addition, we recently found using chromatin immunoprecipitation and fluorescence microscopy that the subset of telomeres that are bound by Rad52 associate with the Nuclear Pore Complexes (NPCs) [59].

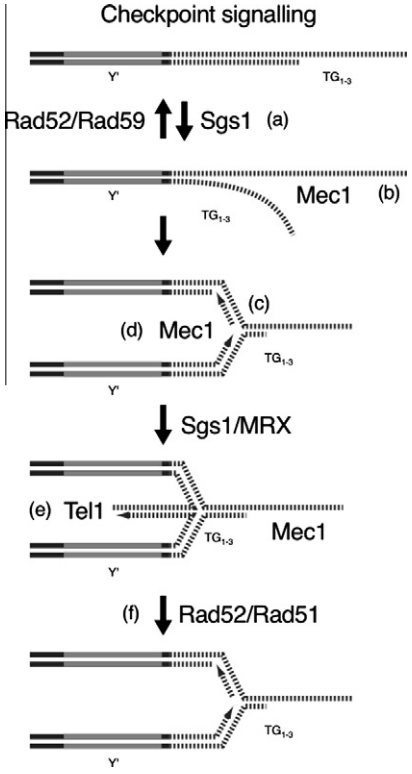

Fig. 2. Checkpoint signalling at eroded telomeres. Both major checkpoint kinases, Mec1 (ATR) and Tel1 (ATM) contribute to the DNA damage response to eroded telomeres. Tel1 is activated by MRX binding to DNA ends and Mec1 is activated by single-stranded DNA in the context of resected ends or stalled replication forks. Uncapped telomeres are potential substrates for unwinding and/or resection by the 3′–5′ helicase Sgs1 and an associated nuclease. (a) The unwinding of uncapped telomeres by Sgs1 may be suppressed by the strand-annealing activity of Rad52 and Rad59 as suggested by accelerated senescence of a *rad52*Δ mutant [83]. Upon unwinding, telomeric single-stranded DNA may activate the Mec1-dependent checkpoint (b). Further, uncapped and resected telomeres (c) will lead to replication fork stalling and activation of Mec1 (d). It is not entirely clear, which structure may be recognized by MRX-Tel1, but it could be either the DNA end of the regressed fork (shown in (e)) or a DNA end formed during replication fork collapse (not shown). Regression of the collapsed replication fork may require Sgs1 and its restart may require Rad51-Rad52-Rad59 (f).

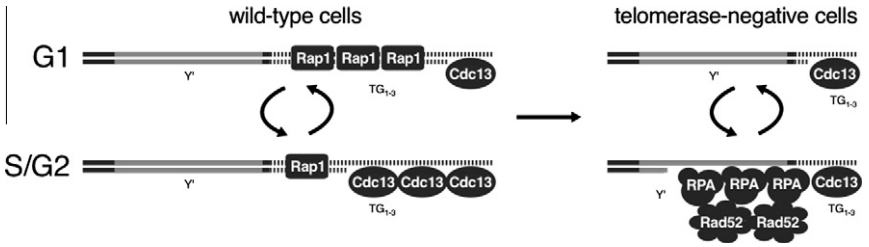

Fig. 3. Controlling recombination at telomeres. Homologous recombination initiates at single-stranded sequences. Single-stranded TG_{1-3} repeats are formed by a burst of resection during S phase. Left: In wild-type cells, single-stranded TG_{1-3} repeats are bound preferentially by Cdc13 to prevent initiation of recombination. Right: Upon telomere shortening in telomerase-negative (*est2*Δ) cells, resection has a greater chance of extending into subtelomeric Y′ elements that are bound preferentially by RPA, which recruits Rad52 to initiate recombination.

Thus eroded telomeres appear to relocate from their membrane anchor site to the NPC. Interestingly, similar localization to the NPC has been reported for collapsed replication forks and persistent DNA damage sites [96]. In an independent study, unrepaired DSBs were found to be enriched at the nuclear periphery in an Mps3-dependent but NPC-independent manner [97]. Based on these observations, the relocalization of telomeres to the NPC in senescing cells could be explained by the increased presence of collapsed replication forks at telomeric regions in the absence of telomerase. Considering that short telomeres do arise in telomerase-proficient cells in vivo [98], and that Rad52 foci are seen at short telomeres in telomerase-proficient cells [59], it is possible that the NPC may also play a role in telomere homeostasis in wild-type cells.

5.3. Regulation of HR at telomeres

Anchoring of telomeres at the nuclear periphery is essential for efficient DSB repair in subtelomeric DNA [99]. Consistently, it was recently reported that spontaneous gene conversion is enhanced in a Nup84- and Slx8-dependent manner by tethering of a donor sequence to the NPC [96]. Further, it was shown that DNA damage, and in particular DSBs, accumulate in mutants of the Nup84 and Nup60 complexes and that mutants of the Nup84 complex are synthetic lethal with genes of the *RAD52* epistasis group [100,101]. These studies are indicative of a role of the NPC in recombinational DNA repair. Since eroded telomeres relocalize to the NPC, we hypothesize that RTE may also be stimulated by NPC association. It was suggested that desumoylation of repair proteins by the SUMO-specific protease Ulp1, which associates with the NPC [102], could be responsible for the observed stimulation of gene conversion [96]. This is supported by changes in sumoylation patterns of RPA, Rad52 and Rad59 observed in nucleoporin mutants and in *slx8*Δ [101,103].

Capping of telomeres is likely the major barrier for recombination at telomere sequences either by inhibiting recombination proteins or by preventing resection of telomeres, which is required to initiate homologous recombination [104,105]. At telomeres, Sae2 and Sgs1 control the two principal pathways for nucleolytic resection of telomere termini [106]. Using a fluorescently-tagged, inducible short telomere, we showed that a single short telomere, which also is the preferred substrate of telomerase [42], was sufficient to recruit recombination proteins [59]. Indeed, a single Rad52- and Cdc13-containing focus is found to colocalize with such a short telomere in 50% of cells [59]. Interestingly, senescing cells in which erosion of all telomeres have progressed approximately synchronously also form just a single Rad52–Cdc13 focus, which colocalizes with one of multiple Rap1/Ku foci. This observation suggests that telomere uncapping and subsequent resection is a local event affecting only one telomere or a cluster of telomeres rather than a global event simultaneously effecting all telomeres in a cell. The number of Cdc13 molecules estimated in the observed foci were equivalent to resection of 140–150 bp of TG_{1-3} sequences assuming that Cdc13 binds 11 nt per molecule [107]. Based on these observations, we propose that a burst of resection at a single telomere marks the transition between recombination-resistant and recombination-prone states at telomeres. Such burst of resection could be explained by a positive feedback loop, where initial resection causes loss of Rap1 leading to further susceptibility to resection and so forth. Alternatively, long telomeres are in a specific conformation, e.g. t-loop or G-quadruplex, which is not supported once telomere length drops below approximately 100 bp.

Interestingly, single-stranded DNA at telomeres does not appear to be sufficient to attract recombination proteins. This was shown by analysing RPA, Rad52 and Cdc13 localization to telomeres with various end structures [59]. In particular, RPA and Rad52 are enriched in Cdc13 foci in *yku70*Δ and *est2*Δ cells, which is indicative of ongoing homologous recombination, but not in temperature-sensitive *cdc17-1* cells, which harbor a mutation in the catalytic subunit of the DNA polymerase I alpha-primase complex that causes telomerase-dependent over-extension of telomeres [108]. Since *yku70*Δ and *cdc17-1* mutants both display single-stranded G-tails, the presence of ssDNA at telomeres is not sufficient for recruiting Rad52. In fact, it appears that recombination is initiated in the *yku70*Δ mutant, because it allows resection to proceed into the subtelomeric X and Y′ elements (Fig. 3).

6. Conclusion

In conclusion, chromosome termini exist in a dynamic state of graded instability defined by the length of the telomere, where long telomeres are stable and invisible to DNA damage machinery and shorter telomeres can be recognized as DSBs or elongated by telomerase. Homologous recombination at telomere sequences is primarily controlled at the level of nucleolytic resection of telomeres during late S phase, and resection into subtelomeric X and Y′ elements, which are not protected by Cdc13, greatly stimulates recruitment of the recombination machinery. However, other factors such at localization of telomeres to the NPC, chromatin remodeling, and sumoylation of recombination proteins may also affect homologous recombination at telomeres although these factors await further studies.

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References

[1] Osterhage, J.L. and Friedman, K.L. (2009) Chromosome end maintenance by telomerase. J. Biol. Chem. 284, 16061–16065.
[2] Greider, C.W. and Blackburn, E.H. (1985) Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell 43, 405–413.
[3] Singer, M.S. and Gottschling, D.E. (1994) TLC1: template RNA component of *Saccharomyces cerevisiae* telomerase. Science 266, 404–409.
[4] Counter, C.M., Meyerson, M., Eaton, E.N. and Weinberg, R.A. (1997) The catalytic subunit of yeast telomerase. Proc. Natl. Acad. Sci. USA 94, 9202–9207.
[5] Lendvay, T.S., Morris, D.K., Sah, J., Balasubramanian, B. and Lundblad, V. (1996) Senescence mutants of *Saccharomyces cerevisiae* with a defect in telomere replication identify three additional EST genes. Genetics 144, 1399–1412.
[6] Lundblad, V. and Szostak, J.W. (1989) A mutant with a defect in telomere elongation leads to senescence in yeast. Cell 57, 633–643.
[7] Lingner, J., Cech, T.R., Hughes, T.R. and Lundblad, V. (1997) Three Ever Shorter Telomere (EST) genes are dispensable for in vitro yeast telomerase activity. Proc. Natl. Acad. Sci. USA 94, 11190–11195.
[8] Cohn, M. and Blackburn, E.H. (1995) Telomerase in yeast. Science 269, 396–400.
[9] Zhou, J., Hidaka, K. and Futcher, B. (2000) The Est1 subunit of yeast telomerase binds the Tlc1 telomerase RNA. Mol. Cell. Biol. 20, 1947–1955.
[10] Lee, J., Mandell, E.K., Tucey, T.M., Morris, D.K. and Lundblad, V. (2008) The Est3 protein associates with yeast telomerase through an OB-fold domain. Nat. Struct. Mol. Biol. 15, 990–997.
[11] Lieber, M.R. (2010) The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. Annu. Rev. Biochem., 79.
[12] San Filippo, J., Sung, P. and Klein, H. (2008) Mechanism of eukaryotic homologous recombination. Annu. Rev. Biochem. 77, 229–257.
[13] Lisby, M. and Rothstein, R. (2009) Choreography of recombination proteins during the DNA damage response. DNA Repair (Amst) 8, 1068–1076.
[14] Clerici, M., Mantiero, D., Guerini, I., Lucchini, G. and Longhese, M.P. (2008) The Yku70-Yku80 complex contributes to regulate double-strand break processing and checkpoint activation during the cell cycle. EMBO Rep. 9, 810–818.
[15] Wu, D., Topper, L.M. and Wilson, T.E. (2008) Recruitment and dissociation of nonhomologous end joining proteins at a DNA double-strand break in *Saccharomyces cerevisiae*. Genetics 178, 1237–1249.
[16] Barlow, J.H., Lisby, M. and Rothstein, R. (2008) Differential regulation of the cellular response to DNA double-strand breaks in G1. Mol. Cell 30, 73–85.
[17] Mimitou, E.P. and Symington, L.S. (2008) Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. Nature 455, 770–774.
[18] Straatman, K.R. and Louis, E.J. (2007) Localization of telomeres and telomere-associated proteins in telomerase-negative *Saccharomyces cerevisiae*. Chromosome Res. 15, 1033–1050.
[19] Lisby, M. and Rothstein, R. (2004) DNA damage checkpoint and repair centers. Curr. Opin. Cell Biol. 16, 328–334.
[20] Nakada, D., Shimomura, T., Matsumoto, K. and Sugimoto, K. (2003) The ATM-related Tel1 protein of *Saccharomyces cerevisiae* controls a checkpoint response following phleomycin treatment. Nucleic Acids Res. 31, 1715–1724.
[21] Lisby, M., Antunez de Mayolo, A., Mortensen, U.H. and Rothstein, R. (2003) Cell cycle-regulated centers of DNA double-strand break repair. Cell Cycle 2, 479–483.
[22] Lobachev, K., Vitriol, E., Stemple, J., Resnick, M.A. and Bloom, K. (2004) Chromosome fragmentation after induction of a double-strand break is an active process prevented by the RMX repair complex. Curr. Biol. 14, 2107–2112.
[23] Lisby, M., Barlow, J.H., Burgess, R.C. and Rothstein, R. (2004) Choreography of the DNA damage response; spatiotemporal relationships among checkpoint and repair proteins. Cell 118, 699–713.
[24] Ira, G., Pellicioli, A., Balijja, A., Wang, X., Fiorani, S., Carotenuto, W., Liberi, G., Bressan, D., Wan, L., Hollingsworth, N.M., Haber, J.E. and Foiani, M. (2004) DNA end resection, homologous recombination and DNA damage checkpoint activation require CDK1. Nature 431, 1011–1017.
[25] Huertas, P., Cortes-Ledesma, F., Sartori, A.A., Aguilera, A. and Jackson, S.P. (2008) CDK targets Sae2 to control DNA-end resection and homologous recombination. Nature 455, 689–692.
[26] Torres-Rosell, J. et al. (2007) The Smc5–Smc6 complex and SUMO modification of Rad52 regulates recombinational repair at the ribosomal gene locus. Nat. Cell Biol. 9, 923–931.
[27] Tsukuda, T., Fleming, A.B., Nickoloff, J.A. and Osley, M.A. (2005) Chromatin remodelling at a DNA double-strand break site in *Saccharomyces cerevisiae*. Nature 438, 379–383.
[28] Sinha, M., Watanabe, S., Johnson, A., Moazed, D. and Peterson, C.L. (2009) Recombinational repair within heterochromatin requires ATP-dependent chromatin remodeling. Cell 138, 1109–1121.
[29] Tsukuda, T., Lo, Y.C., Krishna, S., Sterk, R., Osley, M.A. and Nickoloff, J.A. (2009) INO80-dependent chromatin remodeling regulates early and late stages of mitotic homologous recombination. DNA Repair (Amst) 8, 360–369.
[30] van Attikum, H., Fritsch, O. and Gasser, S.M. (2007) Distinct roles for SWR1 and INO80 chromatin remodeling complexes at chromosomal double-strand breaks. EMBO J. 26, 4113–4125.
[31] Pardo, B. and Marcand, S. (2005) Rap1 prevents telomere fusions by nonhomologous end joining. EMBO J. 24, 3117–3127.
[32] Levy, M.Z., Allsopp, R.C., Futcher, A.B., Greider, C.W. and Harley, C.B. (1992) Telomere end-replication problem and cell aging. J. Mol. Biol. 225, 951–960.
[33] Marcand, S., Brevet, V. and Gilson, E. (1999) Progressive *cis*-inhibition of telomerase upon telomere elongation. EMBO J. 18, 3509–3519.
[34] Tsuji, A., Ishiko, A., Takasaki, T. and Ikeda, N. (2002) Estimating age of humans based on telomere shortening. Forensic Sci. Int. 126, 197–199.
[35] Ren, F., Li, C., Xi, H., Wen, Y. and Huang, K. (2009) Estimation of human age according to telomere shortening in peripheral blood leukocytes of Tibetan. Am. J. Forensic Med. Pathol. 30, 252–255.
[36] Armanios, M., Alder, J.K., Parry, E.M., Karim, B., Strong, M.A. and Greider, C.W. (2009) Short telomeres are sufficient to cause the degenerative defects associated with aging. Am. J. Hum. Genet. 85, 823–832.
[37] Artandi, S.E. and DePinho, R.A. (2010) Telomeres and telomerase in cancer. Carcinogenesis 31, 9–18.
[38] Le, S., Moore, J.K., Haber, J.E. and Greider, C.W. (1999) *RAD50* and *RAD51* define two pathways that collaborate to maintain telomeres in the absence of telomerase. Genetics 152, 143–152.
[39] Reddel, R.R. (2003) Alternative lengthening of telomeres, telomerase, and cancer. Cancer Lett. 194, 155–162.
[40] McEachern, M.J. and Haber, J.E. (2006) Break-induced replication and recombinational telomere elongation in yeast. Annu. Rev. Biochem. 75, 111–135.
[41] Wellinger, R.J., Ethier, K., Labrecque, P. and Zakian, V.A. (1996) Evidence for a new step in telomere maintenance. Cell 85, 423–433.
[42] Teixeira, M.T., Arneric, M., Sperisen, P. and Lingner, J. (2004) Telomere length homeostasis is achieved via a switch between telomerase- extendible and -nonextendible states. Cell 117, 323–335.
[43] Nugent, C.I., Hughes, T.R., Lue, N.F. and Lundblad, V. (1996) Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance. Science 274, 249–252.
[44] Kyrion, G., Boakye, K.A. and Lustig, A.J. (1992) C-terminal truncation of RAP1 results in the deregulation of telomere size, stability, and function in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 12, 5159–5173.
[45] Negrini, S., Ribaud, V., Bianchi, A. and Shore, D. (2007) DNA breaks are masked by multiple Rap1 binding in yeast: implications for telomere capping and telomerase regulation. Genes Dev. 21, 292–302.
[46] Marcand, S., Gilson, E. and Shore, D. (1997) A protein-counting mechanism for telomere length regulation in yeast. Science 275, 986–990.
[47] Garvik, B., Carson, M. and Hartwell, L. (1995) Single-stranded DNA arising at telomeres in cdc13 mutants may constitute a specific signal for the RAD9 checkpoint. Mol. Cell. Biol. 15, 6128–6138.
[48] Grandin, N., Damon, C. and Charbonneau, M. (2001) Ten1 functions in telomere end protection and length regulation in association with Stn1 and Cdc13. EMBO J. 20, 1173–1183.
[49] Gao, H., Cervantes, R.B., Mandell, E.K., Otero, J.H. and Lundblad, V. (2007) RPA-like proteins mediate yeast telomere function. Nat. Struct. Mol. Biol. 14, 208–214.
[50] Sun, J., Yu, E.Y., Yang, Y., Confer, L.A., Sun, S.H., Wan, K., Lue, N.F. and Lei, M. (2009) Stn1-Ten1 is an Rpa2-Rpa3-like complex at telomeres. Genes Dev. 23, 2900–2914.
[51] Zappulla, D.C., Roberts, J.N., Goodrich, K.J., Cech, T.R. and Wuttke, D.S. (2009) Inhibition of yeast telomerase action by the telomeric ssDNA-binding protein, Cdc13p. Nucleic Acids Res. 37, 354–367.
[52] Puglisi, A., Bianchi, A., Lemmens, L., Damay, P. and Shore, D. (2008) Distinct roles for yeast Stn1 in telomere capping and telomerase inhibition. EMBO J. 27, 2328–2339.
[53] Qian, W., Wang, J., Jin, N.N., Fu, X.H., Lin, Y.C., Lin, J.J. and Zhou, J.Q. (2009) Ten1p promotes the telomeric DNA-binding activity of Cdc13p: implication for its function in telomere length regulation. Cell Res. 19, 849–863.
[54] Chandra, A., Hughes, T.R., Nugent, C.I. and Lundblad, V. (2001) Cdc13 both positively and negatively regulates telomere replication. Genes Dev. 15, 404–414.
[55] Marcand, S., Pardo, B., Gratias, A., Cahun, S. and Callebaut, I. (2008) Multiple pathways inhibit NHEJ at telomeres. Genes Dev. 22, 1153–1158.
[56] Hirano, Y., Fukunaga, K. and Sugimoto, K. (2009) Rif1 and rif2 inhibit localization of tel1 to DNA ends. Mol. Cell 33, 312–322.
[57] Chan, S.W. and Blackburn, E.H. (2003) Telomerase and ATM/Tel1p protect telomeres from nonhomologous end joining. Mol. Cell 11, 1379–1387.
[58] Mieczkowski, P.A., Mieczkowska, J.O., Dominska, M. and Petes, T.D. (2003) Genetic regulation of telomere-telomere fusions in the yeast *Saccharomyces cerevisae*. Proc. Natl. Acad. Sci. USA 100, 10854–10859.
[59] Khadaroo, B., Teixeira, M.T., Luciano, P., Eckert-Boulet, N., Germann, S.M., Simon, M.N., Gallina, I., Abdallah, P., Gilson, E., Géeli, V. and Lisby, M. (2009) The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. Nat. Cell Biol. 11, 980–987.
[60] Arneric, M. and Lingner, J. (2007) Tel1 kinase and subtelomere-bound Tbf1 mediate preferential elongation of short telomeres by telomerase in yeast. EMBO Rep. 8, 1080–1085.
[61] Hayflick, L. and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. Exp. Cell Res. 25, 585–621.
[62] Hayflick, L. (1965) The limited in vitro lifetime of human diploid cell strains. Exp. Cell Res. 37, 614–636.

[63] Olovnikov, A.M. (1973) A theory of marginotomy. The incomplete copying of template margin in enzymic synthesis of polynucleotides and biological significance of the phenomenon. J. Theor. Biol. 41, 181–190.
[64] Watson, J.D. (1972) Origin of concatemeric T7 DNA. Nat. New Biol. 239, 197–201.
[65] Cukusic, A., Skrobot Vidacek, N., Sopta, M. and Rubelj, I. (2008) Telomerase regulation at the crossroads of cell fate. Cytogenet. Genome Res. 122, 263–272.
[66] Cristofalo, V.J., Lorenzini, A., Allen, R.G., Torres, C. and Tresini, M. (2004) Replicative senescence: a critical review. Mech. Ageing Dev. 125, 827–848.
[67] Royle, N.J., Foxon, J., Jeyapalan, J.N., Mendez-Bermudez, A., Novo, C.L., Williams, J. and Cotton, V.E. (2008) Telomere length maintenance – an ALTernative mechanism. Cytogenet. Genome Res. 122, 281–291.
[68] Lundblad, V. and Blackburn, E.H. (1993) An alternative pathway for yeast telomere maintenance rescues est1- senescence. Cell 73, 347–360.
[69] Signon, L., Malkova, A., Naylor, M.L., Klein, H. and Haber, J.E. (2001) Genetic requirements for RAD51- and RAD54-independent break-induced replication repair of a chromosomal double-strand break. Mol. Cell. Biol. 21, 2048–2056.
[70] Lydeard, J.R., Jain, S., Yamaguchi, M. and Haber, J.E. (2007) Break-induced replication and telomerase-independent telomere maintenance require Pol32. Nature 448, 820–823.
[71] Teng, S.C. and Zakian, V.A. (1999) Telomere-telomere recombination is an efficient bypass pathway for telomere maintenance in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19, 8083–8093.
[72] Cohen, H. and Sinclair, D.A. (2001) Recombination-mediated lengthening of terminal telomeric repeats requires the Sgs1 DNA helicase. Proc. Natl. Acad. Sci. USA 98, 3174–3179.
[73] Chen, Q., Ijpma, A. and Greider, C.W. (2001) Two survivor pathways that allow growth in the absence of telomerase are generated by distinct telomere recombination events. Mol. Cell. Biol. 21, 1819–1827.
[74] Tsai, Y.L., Tseng, S.F., Chang, S.H., Lin, C.C. and Teng, S.C. (2002) Involvement of replicative polymerases, Tel1p, Mec1p, Cdc13p, and the Ku complex in telomere–telomere recombination. Mol. Cell. Biol. 22, 5679–5687.
[75] Lin, C.Y., Chang, H.H., Wu, K.J., Tseng, S.F., Lin, C.C., Lin, C.P. and Teng, S.C. (2005) Extrachromosomal telomeric circles contribute to Rad52-, Rad50-, and polymerase delta-mediated telomere-telomere recombination in *Saccharomyces cerevisiae*. Eukaryot. Cell 4, 327–336.
[76] Larrivee, M. and Wellinger, R.J. (2006) Telomerase- and capping-independent yeast survivors with alternate telomere states. Nat. Cell Biol. 8, 741–747.
[77] Thrower, D.A., Stemple, J., Yeh, E. and Bloom, K. (2003) Nuclear oscillations and nuclear filament formation accompany single-strand annealing repair of a dicentric chromosome in *Saccharomyces cerevisiae*. J. Cell Sci. 116, 561–569.
[78] IJpma, A.S. and Greider, C.W. (2003) Short telomeres induce a DNA damage response in *Saccharomyces cerevisiae*. Mol. Biol. Cell 14, 987–1001.
[79] Grandin, N., Bailly, A. and Charbonneau, M. (2005) Activation of Mrc1, a mediator of the replication checkpoint, by telomere erosion. Biol. Cell 97, 799–814.
[80] Takai, H., Smogorzewska, A. and de Lange, T. (2003) DNA damage foci at dysfunctional telomeres. Curr. Biol. 13, 1549–1556.
[81] Verdun, R.E., Crabbe, L., Haggblom, C. and Karlseder, J. (2005) Functional human telomeres are recognized as DNA damage in G2 of the cell cycle. Mol. Cell 20, 551–561.
[82] Celli, G.B., Denchi, E.L. and de Lange, T. (2006) Ku70 stimulates fusion of dysfunctional telomeres yet protects chromosome ends from homologous recombination. Nat. Cell Biol. 8, 885–890.
[83] Abdallah, P., Luciano, P., Runge, K.W., Lisby, M., Geli, V., Gilson, E. and Teixeira, M.T. (2009) A two-step model for senescence triggered by a single critically short telomere. Nat. Cell Biol. 11, 988–993.
[84] Ritchie, K.B., Mallory, J.C. and Petes, T.D. (1999) Interactions of TLC1 (which encodes the RNA subunit of telomerase), TEL1, and MEC1 in regulating telomere length in the yeast *Saccharomyces cerevisiae*. Mol. Cell. Biol. 19, 6065–6075.
[85] Enomoto, S., Glowczewski, L. and Berman, J. (2002) 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 13, 2626–2638.
[86] Sabourin, M., Tuzon, C.T. and Zakian, V.A. (2007) Telomerase and Tel1p preferentially associate with short telomeres in *S. cerevisiae*. Mol. Cell 27, 550–561.
[87] Sugawara, N. and Haber, J.E. (1992) Characterization of double-strand break-induced recombination: homology requirements and single-stranded DNA formation. Mol. Cell. Biol. 12, 563–575.
[88] Gotta, M., Laroche, T., Formenton, A., Maillet, L., Scherthan, H. and Gasser, S.M. (1996) The clustering of telomeres and colocalization with Rap1, Sir3, and Sir4 proteins in wild-type *Saccharomyces cerevisiae*. J. Cell Biol. 134, 1349–1363.
[89] Therizols, P., Duong, T., Dujon, B., Zimmer, C. and Fabre, E. (2010) Chromosome arm length and nuclear constraints determine the dynamic relationship of yeast subtelomeres. Proc. Natl. Acad. Sci. USA 107, 2025–2030.
[90] Hediger, F., Neumann, F.R., Van Houwe, G., Dubrana, K. and Gasser, S.M. (2002) Live imaging of telomeres: yKu and Sir proteins define redundant telomere-anchoring pathways in yeast. Curr. Biol. 12, 2076–2089.
[91] Taddei, A., Hediger, F., Neumann, F.R., Bauer, C. and Gasser, S.M. (2004) Separation of silencing from perinuclear anchoring functions in yeast Ku80, Sir4 and Esc1 proteins. EMBO J. 23, 1301–1312.
[92] Schober, H., Ferreira, H., Kalck, V., Gehlen, L.R. and Gasser, S.M. (2009) Yeast telomerase and the SUN domain protein Mps3 anchor telomeres and repress subtelomeric recombination. Genes Dev. 23, 928–938.
[93] Antoniacci, L.M., Kenna, M.A. and Skibbens, R.V. (2007) The nuclear envelope and spindle pole body-associated Mps3 protein bind telomere regulators and function in telomere clustering. Cell Cycle 6, 75–79.
[94] Bupp, J.M., Martin, A.E., Stensrud, E.S. and Jaspersen, S.L. (2007) Telomere anchoring at the nuclear periphery requires the budding yeast Sad1-UNC-84 domain protein Mps3. J. Cell Biol. 179, 845–854.
[95] Hiraoka, Y. and Dernburg, A.F. (2009) The SUN rises on meiotic chromosome dynamics. Dev. Cell 17, 598–605.
[96] Nagai, S., Dubrana, K., Tsai-Pflugfelder, M., Davidson, M.B., Roberts, T.M., Brown, G.W., Varela, E., Hediger, F., Gasser, S.M. and Krogan, N.J. (2008) Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase, Science.
[97] Oza, P., Jaspersen, S.L., Miele, A., Dekker, J. and Peterson, C.L. (2009) Mechanisms that regulate localization of a DNA double-strand break to the nuclear periphery. Genes Dev. 23, 912–927.
[98] Chang, M., Arneric, M. and Lingner, J. (2007) Telomerase repeat addition processivity is increased at critically short telomeres in a Tel1-dependent manner in *Saccharomyces cerevisiae*. Genes Dev. 21, 2485–2494.
[99] Therizols, P., Fairhead, C., Cabal, G.G., Genovesio, A., Olivo-Marin, J.C., Dujon, B. and Fabre, E. (2006) Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J. Cell Biol. 172, 189–199.
[100] Loeillet, S., Palancade, B., Cartron, M., Thierry, A., Richard, G.F., Dujon, B., Doye, V. and Nicolas, A. (2005) Genetic network interactions among replication, repair and nuclear pore deficiencies in yeast. DNA Repair (Amst) 4, 459–468.
[101] Palancade, B., Liu, X., Garcia-Rubio, M., Aguilera, A., Zhao, X. and Doye, V. (2007) Nucleoporins prevent DNA damage accumulation by modulating Ulp1-dependent sumoylation processes. Mol. Biol. Cell 18, 2912–2923.
[102] Takahashi, Y., Mizoi, J., Toh, E.A. and Kikuchi, Y. (2000) Yeast Ulp1, an Smt3-specific protease, associates with nucleoporins. J. Biochem. 128, 723–725.
[103] Burgess, R.C., Rahman, S., Lisby, M., Rothstein, R. and Zhao, X. (2007) The Slx5-Slx8 complex affects sumoylation of DNA repair proteins and negatively regulates recombination. Mol. Cell Biol. 27, 6153–6162.
[104] Grossi, S., Bianchi, A., Damay, P. and Shore, D. (2001) Telomere formation by rap1p binding site arrays reveals end-specific length regulation requirements and active telomeric recombination. Mol. Cell Biol. 21, 8117–8128.
[105] DuBois, M.L., Haimberger, Z.W., McIntosh, M.W. and Gottschling, D.E. (2002) A quantitative assay for telomere protection in *Saccharomyces cerevisiae*. Genetics 161, 995–1013.
[106] Bonetti, D., Martina, M., Clerici, M., Lucchini, G. and Longhese, M.P. (2009) Multiple pathways regulate 3′ overhang generation at *S. cerevisiae* telomeres. Mol. Cell 35, 70–81.
[107] Mitton-Fry, R.M. and Wuttke, D.S. (2002) 1H, 13C and 15N resonance assignments of the DNA-binding domain of the essential protein Cdc13 complexed with single-stranded telomeric DNA. J. Biomol. NMR 22, 379–380.
[108] Carson, M.J. and Hartwell, L. (1985) CDC17: an essential gene that prevents telomere elongation in yeast. Cell 42, 249–257.