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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.