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The Mec1p and Tel1p checkpoint kinases allow humanized yeast to tolerate chronic telomere dysfunctions by suppressing telomere fusions

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ABSTRACT

In this work we report that budding yeasts carrying human-type telomeric repeats at their chromosome termini show a chronic activation of the Rad53-dependent DNA damage checkpoint pathway and a G2/M cell cycle delay. Furthermore, in the absence of either *TEL1/ATM* or *MEC1/ATR* genes, which encodes phosphatidylinositol 3-kinase-related kinases (PIKKs), we detected telomere fusions, whose appearance correlates with a reduced cell viability and a high rate of genome instability. Based on sequence analysis, telomere fusions occurred primarily between ultrashort telomeres. Microcolony formation assays argue against the possibility that fusion-containing cells are eliminated by PIKK-dependent signalling.

These findings reveal that humanized telomeres in yeast cells are sensed as a chronically damaged DNA but do not greatly impair cell viability as long as the cells have a functional DNA damage checkpoint.

1. Introduction

Telomeres are the physical ends of linear chromosomes and contribute to genome integrity. Due to their terminal position, telomeres require a special mechanism to distinguish them from DSBs. Thus, telomeres are either hidden from DNA damage checkpoints or they are detected as DSBs but processed differently [1].

In both yeasts and mammals, several factors involved in DNA damage response also have roles in telomere metabolism. For example, the Ku70/Ku80 heterodimer and the Mre11-Rad50-Xrs2 (MRX) complex are positive regulators of telomere length as demonstrated by telomere shortening in null mutants [2,3]. In particular, Tel1 is required for the maintenance of the steady state telomere length and for the activity of the Rap1-dependent counting mechanism [4,5]. Mec1 also contributes to regulation of telomere length but acts in a different epistasis group from that of MRX and Tel1. In fact, *mec1 tel1* cells, as well as *mec1* cells lacking any of the MRX proteins, have a defect in telomerase-mediated telomere lengthening [6,7].

Phosphoinositide 3-kinase (PIKK proteins) family members are primary sensors of DNA damage. This family includes the *Saccharomyces cerevisiae* Mec1p and Tel1p and the mammalian ATR and ATM proteins. These checkpoint kinases activate a protein kinase cascade to regulate downstream effector kinases such as Rad53 and Chk1. Sensing and signalling to downstream effectors are coupled by the Rad9 adaptor, which activates Rad53 and Chk1 by distinct and independent mechanisms [8,9]. In *S. cerevisiae* DNA damage is mainly sensed by Mec1p, whereas Tel1p seems to play a secondary role [10]. Both Mec1p and Tel1p are important for telomerase-mediated telomere maintenance, with Tel1p playing the major role. In fact, although *mec1-21* allele is synthetically senescent with *tel1* [6], *tel1* cells have much shorter telomeres than *mec1-21* cells, whose telomeres are only slightly shorter than WT. Interestingly it has been shown that Tel1p can phosphorylate Cdc13 *in vitro* [11] suggesting a role for it in telomerase recruitment. Consistent with this hypothesis is the finding that Tel1p helps recruitment of telomerase in late S/G2 whereas Mec1p does not appear to be involved in this process [12] and that short telomeres, which are preferentially elongated with respect to full-length telomeres [13–15] are more avidly bound by Est1p, Est2p and Tel1p [16–18]. Moreover, it has recently shown that the preferential elongation of short telomeres require Tel1p, although a Tbf1p-mediated backup mechanism has been described in *tel1* mutants [19].

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Baker's yeast telomeres are organized into a complex nucleoprotein structure consisting of a terminal tract of ~300 bps of duplex TG_{1-3} repeats and a short 3′ single strand overhang. This overhang, which is present throughout the cell cycle, increases in length in late S phase and is bound *in vivo* by Cdc13p, a TG_{1-3} sequence specific binding protein. All yeast telomeres also have the subtelomeric X element and about half have up to four tandem copies of the Y′ subtelomeric element distal to X. Short tract of telomeric repeats are often found between the Y′ and X elements. Telomeric DNA is assembled into a non nucleosomal structure, whose main component is Rap1p, a duplex, sequence specific DNA binding protein that binds telomeric DNA with its Myb-like binding domain and tethers Rif1 and Rif2 by interactions with the C-terminal domain [20]. Deletion of the C-terminal part of Rap1p or of Rif1p and/or Rif2p, causes telomerase-dependent telomere lengthening, suggesting that Rap1p and its associated proteins act negatively on telomere length [21,22]. The amount of telomeric DNA and therefore of Rap1 and the Rif proteins negatively regulates telomerase-mediated lengthening as short telomeres are preferentially elongated. Many yeast telomeric proteins are conserved in evolution such as the human factors hRap1, TRF1 and TRF2 are part of human telomeres and act as negative regulators of telomere length [23].

Conservation of telomere structure and associated factors between human and yeast opens a new possibility to study human telomeres in a more amenable genetic system such as budding yeast, which may allow the discovery of unknown mechanisms related to telomere replication, protection and maintenance. Yeast telomeres that have the same sequence as human telomeres, can be generated by using a "humanized" version of TLC1, the tlc1-h allele, in which the template region is altered so that it encodes T_2AG_3 repeats [24]. By using this strain *de novo* telomere formation approach was used to generate a telomere composed solely of T_2AG_3 repeats and devoid of Rap1p and the Rif proteins [25,26]. Since this "humanized" telomere has normal mitotic stability and a properly regulated length, *RAP1* is not essential for the maintenance of the yeast telomeres and to regulate their length [25,26].

It was shown that the insertion of all 63 mutant combinations of telomeric template of the RNA component of yeast telomerase caused incorporation or misincorporation of the template mutations within telomeres [27]. Although mutants exhibited a variety of growth and telomeric defects, all of them showed aberrant chromosome separation and segregation, suggesting activation of the DNA damage checkpoint response. However, deletion of the *DDC1*, *MEC3* or *DDC2* checkpoint genes failed to rescue this defect, suggesting that telomeres activate alternative checkpoint responses [27]. We and others have previously demonstrated that yeast cells containing the human telomerase RNA template have telomeres that are a hybrid of yeast and human telomeric DNA that apparently protect the chromosomal ends accurately [25,26], even in the absence of any residual yeast telomeric repeat (solo-vertebrate telomere). By ChIP analysis, it has been demonstrated that the solo-vertebrate telomeres are almost free of Rap1p, whereas the hybrid yeast-human telomeres are still bound by Rap1p due to the presence of an internal core of yeast repeats. Both the solo-vertebrate and the hybrid telomeres are bound by the normally subtelomeric protein Tbf1 that binds T_2AG_3 repeats *in vitro* [26]. These telomeres also bind Cdc13p, consistent with the *in vitro* binding properties of this protein [28,29]. In fact, by ChIP analysis, there is more Cdc13p associated with humanized telomeres than with wt telomeres. This result in addition to the detection of single-stranded T_2AG_3 at the ends of HY chromosomes by non-denaturing hybridization analysis [30] suggested that G-tails on HY telomeres are likely longer and/or present throughout a larger fraction of the cell cycle than in WT cells. Moreover, since both the Cdc13p ChIP and the non-denaturing Southern hybridization analysis show increases over time, telomeric G-tails are likely to increase in length with growth. Thus, the chromatin structure of telomeres in *tlc1-h* cells is very different from that in WT yeast. In the present work, we show that yeast cells with humanized telomeres were delayed in their progression through the cell cycle, often exhibiting a G2/M delay. In addition, the checkpoint kinase Rad53p was chronically active, and this activation was *RAD9*, *MEC1* and *TEL1* dependent. Moreover, the absence of either Mec1p or Tel1p triggered the formation of telomere fusions between ultrashort telomeres and reduced cell viability. These findings reveal that humanized telomeres in yeast cells are sensed as a chronically damaged DNA but do not greatly impair cell viability as long as the cells have a functional DNA damage checkpoint.

2. Materials and methods

2.1. Yeast strains

The yeast strains (Supplementary Table 1) used in this work derive from C6.1 [31] by standard yeast genetics; TLC1 (Y) and tlc1-h (HY) strains have been selected by PCR as described in Brevet at al [25]; null HY and Y *ctf8, ddc1, dun1, lif1, mec1, rad17, rad50, rad51, rad9, sml1* and *tel1* mutants were obtained by KanMX, CloNat and Hyg inserting cassettes as described in Goldstein and McCusker [32]. The inserting fragments were amplified from plasmids containing the corresponding markers (kindly provided by EUROSCARF; http://www.uni-frankfurt.de/fb15/mikro/euroscarf/). All disruptions have been controlled by specific upstream and downstream PCR reactions. To account for telomeric sequence variation during growth, null mutants were obtained from HY clones at two stages: P5 (5 plate passage after sporulation) and P30 (30 plate passages from sporulation) except for *HY tel1* mutants which derived from sporulation of an heterozygous diploid (*TLC1*/*tlc1-h*, *TEL1*/*tel1*::KanMX) and subsequent plate passages. Null mutants were re-streaked five times prior analysis to adapt telomere length to the new genotype. Plates have been incubated 2–4 days at 30 °C until colonies reached 2–3 mm, each plate passage was estimated to correspond to about 20 generations.

2.2. Telomere fusion detection

Telomere fusions (TFs) were detected by PCR as describe in Mieczkowski et al. [33]. 10–25 independent cultures for each strain have been tested. First, each DNA preparation was amplified with actin primers to account for DNA quality. Limiting dilution PCR [34]: the relevant genomic DNA, diluted with genomic DNAs from a WT strain (W303) to maintain the total amount of DNA constant to 50 ng, was amplified with primers X and Y; the PCR products were run on agarose gels and the intensity of the TFs bands was determined. TFs quantification: the intensity of the TF bands, obtained from PCR reactions with 50 ng genomic DNA, was normalized to that of the endogenous actin gene from the same reaction. TF positive control consists of a cloned telomeric fusion 400 bp in size. PCR products were separated by electrophoresis using standard agarose gels and high sensitive FlashGels (Cambrex). The intensity of the bands was determined by Kodak1D software.

2.3. Telomere and TFs cloning

Hybrid yeast-human telomeric fragments were amplified by PCR with primers matching the X and Y' elements (1L and 5R) and the human telomeric repeat (A_2TC_3); primers are provided in Supplementary Table 2. DNA extracts from three independent cultures (each inoculated with a single colony) for each strain,