Supplementary data

Telomerase-dependent generation of 70 nt long telomeric single-stranded 3’ overhangs in yeast.

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Supplementary methods

Pre-treatment of genomic DNA with T7 exonuclease.
The T7 exonuclease specifically digests DNA in the 5’ to 3’ direction, resulting in the elongation of telomeric single-stranded 3’ overhangs. Genomic DNA (5 µg) of S. castellii strain Y320 was incubated with 7 U of T7 exonuclease (NE BioLabs) for 90 seconds at 24°C (30 µl reaction volume; 50 mM Tris-HCl pH 8.0, 5 mM MgCl₂, 1 mM DTT). The reaction was stopped by the addition of 20 µl 10 mM Tris-HCl pH 8, 1 mM EDTA (TE) and 50 µl of phenol:chloroform and the DNA was extracted and then precipitated with isopropanol. The purified DNA was dissolved in TE and then digested with 1 U duplex-specific nuclease (DSN) as in the DSN assay described in the Materials and Methods.

Supplementary legends

Figure S1. Synchronization of S. castellii cell growth. (A) After 4 hours of incubation with 150 mM hydroxyurea (HU) the characteristic dumbbell shape was obtained for 95% of the cells, indicating an efficient block in the S phase of the cell cycle. (B) Flow cytometry analysis of propidium iodide stained cells show that the cells are synchronously growing after the release from the HU block. Cells were blocked by HU in the early S phase, released into fresh medium, and sampled every 10 minutes. Pre: Before addition of HU; Block: after 4 hrs HU treatment; 0 min: at the time of release.

Figure S2. Treatment of S. castellii genomic DNA with T7 exonuclease results in a higher signal of the telomeric 3’ overhangs. Genomic DNA from S. castellii Y320 was digested with T7 exonuclease prior to the DSN assay. T7 Exo + or - denote with or without exonuclease pre-treatment. DNA Molecular size markers are indicated in nucleotides: lane 1, telomere oligonucleotide markers; lane 4, GeneRuler molecular size marker.

Figure S3. The fraction of 70 nt 3’ overhangs are generated in S phase. S. castellii Y320 cells were synchronized by hydroxyurea (HU) treatment and released into S phase. Samples for DNA preparation and DSN assay were collected just before addition of HU (Pre), at the time of release from the HU block (0) and then at every 10 minutes. Time course experiments were performed for a total of 300 minutes (A) and 120 minutes (B), respectively. Size markers are indicated in nucleotides. The size windows used for quantification of the signals are indicated to the right; S, Short 20-40 nt ; M, Medium 40-60 nt; L, Long 60-90 nt; UL, Ultra-Long 90-200 nt.
Figure S4. A minor fraction of the cells move in synchrony for more than one cell cycle. Flow cytometry analysis of propidium iodide stained *S. castellii* Y320 cells. The cells were synchronized by hydroxyurea (HU) treatment and released into S phase. Samples were collected just before addition of HU (Pre), at the time of release from the HU block (0), and then at every 10 minutes for a total of 130 min.

Supplementary figures

Figure S1A
Figure S1B
Figure S2
Figure S3
Figure S4