View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Telomere maintenance is dependent on activities required for end repair of double-strand breaks

Constance I. Nugent*, Giovanni Bosco†, Lyle O. Ross*, Sara K. Evans‡, Andrew P. Salinger*, J. Kent Moore†, James E. Haber† and Victoria Lundblad*‡

Telomeres are functionally distinct from ends generated by chromosome breakage, in that telomeres, unlike double-strand breaks, are insulated from recombination with other chromosomal termini [1]. We report that the Ku heterodimer and the Rad50/Mre11/Xrs2 complex, both of which are required for repair of double-strand breaks [2–5], have separate roles in normal telomere maintenance in yeast. Using epistasis analysis, we show that the Ku end-binding complex defined a third telomere-associated activity, required in parallel with telomerase [6] and Cdc13, a protein binding the single-strand portion of telomere DNA [7,8]. Furthermore, loss of Ku function altered the expression of telomere-located genes, indicative of a disruption of telomeric chromatin. These data suggest that the Ku complex and the Cdc13 protein function as terminus-binding factors, contributing distinct roles in chromosome end protection. In contrast, *MRE11* and *RAD50* were required for the telomerase-mediated pathway, rather than for telomeric end protection; we propose that this complex functions to prepare DNA ends for telomerase to replicate. These results suggest that as a part of normal telomere maintenance, telomeres are identified as double-strand breaks, with additional mechanisms required to prevent telomere recombination. Ku, Cdc13 and telomerase define three epistasis groups required in parallel for telomere maintenance.

Addresses: *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, USA. †Brandeis University, Rosenstiel Center, Waltham, Massachusetts 02254-9110, USA. ‡Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, USA.

Correspondence: James Haber and Victoria Lundblad
E-mail: haber@hydra.rose.brandeis.edu and lundblad@bcm.tmc.edu

Received: **11 March 1998**
Revised: **20 April 1998**
Accepted: **20 April 1998**

Published: **11 May 1998**

Current Biology 1998, 8:657–660
http://biomednet.com/elecref/0960982200800657

© Current Biology Ltd ISSN 0960-9822

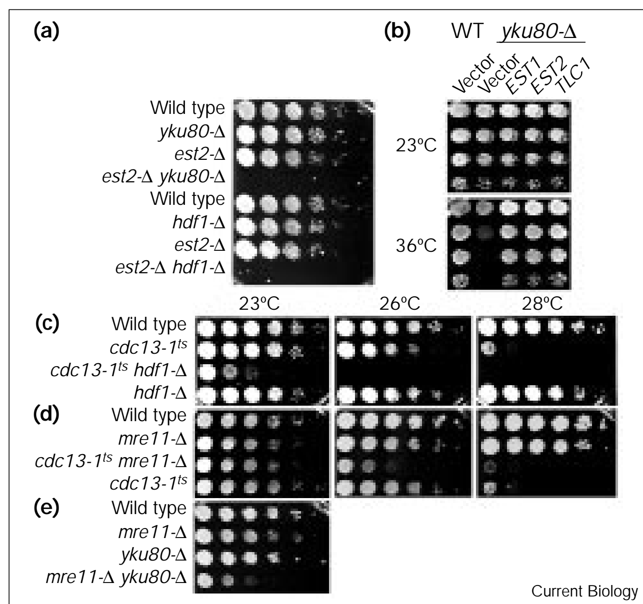
Results and discussion

In *Saccharomyces cerevisiae*, telomere replication depends upon the telomerase RNA gene *TLC1* [9], the catalytic subunit *EST2* [10,11], and three other genes, *EST1*, *EST3* and *EST4/CDC13* (reviewed in [6]). Mutations in all five genes result in senescence, whereby the telomeric repeat tract becomes progressively shorter until telomere function is lost, resulting in chromosome loss and cell death. *CDC13* has also been proposed to separately contribute to telomere end protection in yeast, as the loss of *CDC13* activity that occurs in a *cdc13-1*^{ts} strain results in rapid degradation of the C-rich strand of the telomere and a consequent loss of telomere integrity [12]. Telomere end protection and telomere replication appear to be functionally distinct activities; thus, a telomerase-defective *cdc13-1*^{ts} double mutant strain has an exaggerated growth defect relative to either single mutant strain [7].

We designed two complementary genetic screens to identify additional genes required for telomere replication or integrity. Screen A probed for mutations that lowered the maximum permissive temperature of a *cdc13-1*^{ts} strain, and screen B identified mutations that enhanced the senescence phenotype of an *est1-Δ* mutant strain. As expected, one mutation in *EST1* and two mutations in *EST2* were isolated in screen A that, in combination with the *cdc13-1*^{ts} allele, resulted in enhanced temperature sensitivity and exaggerated senescence. Surprisingly, both screens revealed mutations in genes required for repair of DNA double-strand breaks. Screen A yielded two mutations each in *YKU80* and *RAD50*, and screen B identified two mutations in *YKU80*. Both the Ku heterodimer (encoded by the *YKU80* and *HDF1* genes) and the multiprotein Rad50/Mre11/Xrs2 complex have been shown to be critical for non-homologous DNA double-strand break end-joining repair (NHEJ) [2–5]. Ku binds in a sequence-independent manner with high affinity to the ends of duplex DNA as well as to nicks in double-stranded DNA [3,13]. The precise biochemical function of the Rad50/Mre11/Xrs2 complex in NHEJ is less well understood, with evidence in support of an enzymatic role as a 5′ to 3′ exonuclease [14,15], or alternatively a more structural role [16].

We then performed directed epistasis tests by examining the phenotypes of strains carrying various mutant combinations, which demonstrated that telomerase, the Ku complex and Cdc13p each contribute distinct roles at the telomere. Any combination of Ku⁻ null mutations with deletions of *EST1*, *EST2* or *TLC1* resulted in an exacerbated phenotype, as the double mutant strains all exhibited accelerated inviability (Figure 1a and data not shown). Haploid *hdf1-Δ est2-Δ* and *yku80-Δ est2-Δ* double mutant spores generated from heterozygous diploids gave rise to colonies that consisted of mostly inviable cells, so that

Figure 1



The Ku heterodimer, telomerase and Cdc13 protein are each required for full telomere function. **(a)** Growth of isogenic haploid *hdf1-Δ* and *yku80-Δ* mutant strains in the presence or absence of an *est2-Δ* mutation, using equivalent numbers of cells taken directly from colonies off the dissection plate of freshly dissected isogenic diploid strains (after ~25 generations of growth). Combinations of *yku80-Δ* and *hdf1-Δ* with *est1-Δ*, *tlc1-Δ* and *cdc13-2^{est}* mutations were also tested, with results identical to those shown for *est2-Δ* (data not shown). **(b)** Temperature lethality of *yku80-Δ* (or *hdf1-Δ*; data not shown) is suppressed by increased expression of *EST1*, *EST2* or *TLC1*. Wild-type (WT) or *yku80-Δ* strains were transformed with: vector alone (pVL399), pVL784 (2μ *LEU2* p*ADH-EST1*), pVL999 (2μ *LEU2* p*ADH-EST2*), or pVL799 (2μ *LEU2* p*ADH-TLC1*). Cells were grown in selective media and examined at 23°C and 36°C after sufficient growth to allow manifestation of the Ku-associated temperature-sensitive phenotype. **(c)** Growth of the haploid *hdf1-Δ* strain in the presence or absence of the *cdc13-1^{ts}* allele, with phenotypes assessed after ~25 generations of growth of freshly dissected isogenic diploid strains; *yku80-Δ cdc13-1^{ts}* double mutants display a similar phenotype (data not shown). **(d)** The *mre11-Δ* strain shows a synthetic phenotype in combination with *cdc13-1^{ts}* (incubated at 23°C, 26°C and 28°C). **(e)** The *mre11-Δ* strain exhibits a synthetic phenotype in combination with *yku80-Δ*.

such colonies could not be further propagated (Figure 1a). In contrast, *est2-Δ* mutant spores initially exhibited growth comparable to that of wild type, followed by a progressive decrease in cell viability (that is, senescence) [17]. The simplest explanation for this synthetic near-lethality is that increased telomere shortening occurs as a consequence of different mutations impacting on two separate telomere length maintenance pathways. Thus, the telomere shortening defect in Ku-deficient cells is not due to loss of telomerase function, but rather to the loss of another activity required to maintain telomere length. Cells devoid of Ku function show a temperature-sensitive growth phenotype with a phenotypic lag, whereby cells proliferate for a limited number of generations at high temperature before they die [18]. This temperature-sensitive phenotype of *yku80* mutants could be suppressed by increased expression of *EST1*, *EST2* or *TLC1* (Figure 1b). Therefore, both increases and decreases in the levels of three genes specifically required for telomerase function alter the growth phenotype of mutations in *HDF1* and *YKU80*.

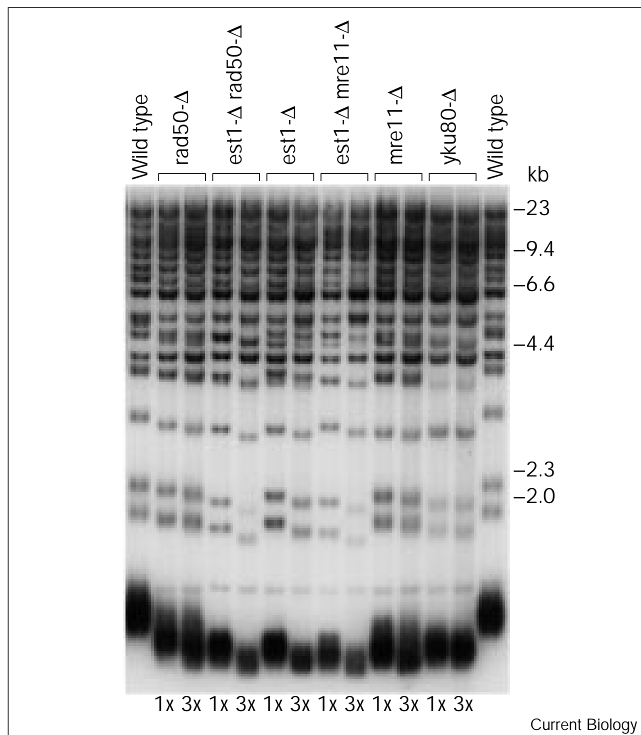
We next examined the effects of combining Ku deletions with the *cdc13-1^{ts}* mutation. As previously observed for *cdc13-1^{ts} tlc1-Δ* double mutants [7], *cdc13-1^{ts} hdf1-Δ* and *cdc13-1^{ts} yku80-Δ* double mutant strains exhibited substantially impaired growth. The maximal permissive temperature of both double mutant strains was reduced relative to the single *cdc13-1^{ts}* strain, and even at permissive temperature, the double mutants had a noticeable growth defect (Figure 1c and data not shown). This synthetic phenotype is not simply the consequence of loss of a Ku-mediated DNA repair pathway required to repair damage generated in the *cdc13-1^{ts}* strain, because a *sir3-Δ* mutation that also eliminates the same DNA end-joining repair pathway [5], did not enhance the defect of a *cdc13-1^{ts}* mutation (data not shown). Therefore, Cdc13p and the Ku proteins, capable of binding single-strand and double-strand DNA substrates, respectively, have separable roles that contribute to telomere integrity.

MRE11 and *RAD50* function in the telomerase pathway

The Ku complex and Rad50/Mre11/Xrs2 are both required for efficient DNA end joining and function in a single epistasis group with respect to DNA end joining in yeast [2–5]. Although previous work has shown that deletion of either of these two groups of genes also results in short telomeres [4,19,20], we show here that these genes have strikingly different roles in telomere maintenance. In contrast to *est2-Δ yku80-Δ* and *est2-Δ hdf1-Δ* strains (Figure 1a), mutations in *MRE11* and *RAD50* failed to enhance the telomere replication defect of telomerase mutants. The *mre11-Δ* and *rad50-Δ* strains showed gradual telomere shortening, although the defect was less severe than that observed in *est1-Δ* mutant strains (Figure 2), and *mre11-Δ* and *rad50-Δ* mutations did not confer a senescence phenotype in our strain background (although *rad50-Δ* strains have been reported to exhibit senescence by others [20]). Double mutant strains combining *mre11-Δ* or *rad50-Δ* with either *est1-Δ* or *est2-Δ* mutations did not result in an enhanced loss of either telomere length or cell viability compared to *est1-Δ* or *est2-Δ* single mutant strains (Figure 2 and data not shown). Thus, by these genetic criteria, both Mre11p and Rad50p function in the telomerase-mediated pathway for telomere replication.

Placement of *MRE11* and *RAD50* in the telomerase epistasis group also predicts that mutations in these two genes should behave the same as mutations in *EST1*, *EST2* and *TLC1* with respect to the two other telomere-specific epistasis groups. Consistent with this expectation, *mre11-Δ*

Figure 2



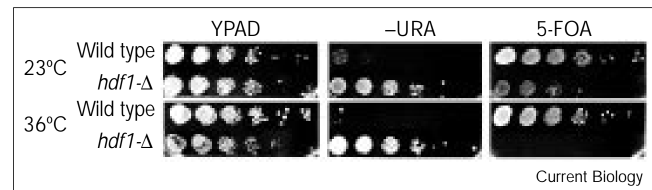
RAD50 and *MRE11* are in the telomerase epistasis group. Comparison of telomere length of isogenic haploid strains after increasing population doublings, examined after ~25 generations (1×) and ~45 generations (3×). The broad 1.2–1.5 kb band represents roughly two-thirds of the telomeres in this strain, and the four bands ranging from 1.8 kb to ~4.0 kb correspond to individual telomeres.

and *rad50-Δ* mutations exhibited enhanced phenotypes with either *cdc13-1*^{ts} mutations or Ku^{-} mutations. Introduction of a *mre11-Δ* or *rad50-Δ* mutation into a *cdc13-1*^{ts} strain reduced the maximum permissive temperature (Figure 1d and data not shown). Similarly, double mutant combinations of *mre11-Δ* or *rad50-Δ* with *hdf1-Δ* or *yku80-Δ* have decreased viability (Figure 1e and data not shown), although the synthetic defect is not as severe as for *est2-Δ yku80-Δ* double mutants (Figure 1a).

Absence of Ku function relieves repression of telomere-located genes

Telomere-localized reporter genes are subject to reversible transcriptional repression [1], referred to as telomeric position effect (TPE). In addition to structural components of telomeric chromatin that are required for TPE, genetic analysis has predicted the existence of a terminus-specific binding factor that is critical for TPE [21]. Three candidates for terminus-specific activities are Cdc13p, the Ku heterodimer, and telomerase itself. We therefore examined the effects of mutations in each of these epistasis groups for effects on TPE. Expression of the *URA3* gene when placed next to the telomere can be monitored via either

Figure 3



The Ku heterodimer is required for silencing of a telomere-located gene. The extent of repression of a telomeric *URA3* reporter gene [9] was measured by plating serial 10-fold dilutions of cells from wild-type and *hdf1-Δ* cells, assessed immediately after dissection of an *HDF1*/*hdf1-Δ* diploid strain, on complete media (YPAD) to monitor total cell viability, on media lacking uracil (–URA) to assess the extent of derepression of *URA3* transcription, and on media containing 5-FOA to determine the proportion of cells able to repress *URA3* transcription. Plates were incubated at either 23°C (5 days) or 36°C (2.5 days).

growth in the absence of uracil or growth in the presence of a drug inhibitory to Ura^{+} cells (5-fluoro-orotic acid, 5-FOA) [9]. Elimination of telomerase function by deletion of *TLC1* or *EST2* or by mutating the telomerase function of *CDC13* (*cdc13-2*^{est}) [7,17] did not alter the level of repression of *URA3* compared to the complete derepression that occurs in the absence of the Sir3 protein (Supplementary material and data not shown). Similarly, *mre11-Δ* and *rad50-Δ* deletions showed no effects on TPE (Supplementary material), consistent with the placement of these two genes in the telomerase epistasis group. Moreover, *cdc13-1*^{ts}, which disrupts a telomere-binding function distinct from the telomerase defect of the *cdc13-2*^{est} allele [7,12], did not alter TPE at either permissive or semi-permissive temperatures ([8] and data not shown).

In contrast, TPE is substantially altered by the loss of the Ku heterodimer. At temperatures permissive for long-term growth (23°C), elimination of Ku resulted in an intermediate effect on *URA3* expression (Figure 3), suggesting that the repressed state is not adequately maintained. An even more severe defect was observed when TPE was examined in an *hdf1-Δ* strain immediately after transfer to 36°C, when the strain was still viable: repression of the telomere-located *URA3* reporter gene was now completely abolished (Figure 3), comparable to results observed when an essential component of telomeric chromatin, Sir3p, is deleted. Thus, the Ku complex appears to play a crucial role in maintaining telomeric chromatin structure, consistent with the prediction for a telomere end-binding activity. This role for Ku is partially redundant at low temperatures, however, with some other unidentified activity at the telomere that is itself temperature-labile.

Our results demonstrate that Ku proteins define a discrete telomere-dependent function that is required in parallel with *CDC13* and telomerase. We further propose that the Ku complex and Cdc13p are terminus-specific proteins

that collaborate to protect the end of the chromosome, and that the inability of a telomerase-defective strain to replicate the terminus, when combined with loss of the activity of Cdc13p or Ku, is catastrophic for telomere maintenance. Several observations support the proposal of two activities required for end protection. First, the biochemical properties of both proteins are consistent with an *in vivo* role in terminus binding: Ku has an affinity for duplex DNA ends [13], whereas Cdc13 binds to single-stranded $G_{1-3}T$ DNA [7,8]. Second, mutations in both lead to alterations in telomeric end structure: loss of *CDC13* function results in removal of the C-rich strand of the telomere and consequent lethality [12], and regulation of the S-phase-specific chromosomal end structure is disrupted in cells that lack Ku function [22]. Loss of Ku function also alters the expression of telomeric reporter genes ([4,23]; this work), as predicted for a telomere end-binding protein [21]. This proposed role for the Ku heterodimer at the telomere is also distinct from that of the Sir complex. Although Sir^- mutations also derepress TPE and DNA joining [1,5], and Hdf1p interacts with Sir4p [5], *sir* mutants do not enhance the temperature-sensitivity of either *hdf1* or *cdc13-ts* mutations (data not shown).

In contrast, the Rad50/Mre11/Xrs2 complex appears to play a role in mediating replication of telomeres via the telomerase pathway. That they are in the same epistasis group as telomerase suggests that they may function to prepare or present DNA ends to telomerase for further replication. Based on a reduced rate of 5′ to 3′ exonucleolytic strand processing of DNA ends with *rad50*, *mre11* and *xrs2* mutations [14,15], this complex has been proposed to have exonuclease activity; if so, these proteins may prepare a single-strand substrate that can be acted upon by telomerase, as telomerase cannot extend a duplex blunt end.

These results indicate that gene products previously implicated in repairing double-strand breaks are also directly involved at another terminus, the telomere. However, a critical difference is that telomeres do not normally allow recombination or end-to-end joining with other chromosome ends. Additional telomere-specific factors, such as the Cdc13 protein, may alter the roles of these proteins when present at the telomere. Further characterization of these proteins in both double-strand break repair and telomere function will be necessary to reveal the similarities and differences in how these two different types of DNA ends are processed.

Supplementary material

A figure showing that neither Cdc13p nor telomerase are required for silencing of a telomere-located gene and additional methodological details are published with this article on the internet.

Acknowledgements

C.I.N., G.B. and L.O.R. contributed equally to this work. The authors would like to thank Alison Bertuch and Sang Eun Lee for stimulating discussions and Nathan Walcott for superb technical support. This work was supported by postdoctoral fellowships from the US Army and Materiel Command (C.I.N.) and the NIH (L.O.R.), and grants from the NIH (GM55867; V.L.) and DOE (91ER61235; J.E.H.).

References

1. Zakian VA: **Structure, function, and replication of *Saccharomyces cerevisiae* telomeres.** *Annu Rev Genet* 1996, **30**:141-172.
2. Moore JK, Haber JE: **Cell cycle and genetic requirements of two pathways of nonhomologous end-joining repair of double-strand breaks in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1996, **16**:2164-2173.
3. Milne GT, Jin S, Shannon KB, Weaver DT: **Mutations in two Ku homologs define a DNA end-joining repair pathway in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1996, **16**:4189-4198.
4. Boulton SJ, Jackson SP: **Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing.** *EMBO J* 1998, **17**:1819-1828.
5. Tsukamoto Y, Kato J, Ikeda H: **Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*.** *Nature* 1997, **388**:900-903.
6. Nugent CI, Lundblad V: **The telomerase reverse transcriptase: components and regulation.** *Genes Dev* 1998, **12**:1073-1085.
7. Nugent CI, Hughes TR, Lue NF, Lundblad V: **Cdc13p: a single-strand telomeric DNA-binding protein with a dual role in yeast telomere maintenance.** *Science* 1996, **274**:249-252.
8. Lin JJ, Zakian VA: **The *Saccharomyces* CDC13 protein is a single-strand TG1–3 telomeric DNA-binding protein *in vitro* that affects telomere behavior *in vivo*.** *Proc Natl Acad Sci USA* 1996, **93**:13760-13765.
9. Singer MS, Gottschling DE: **TLC1: template RNA component of *Saccharomyces cerevisiae* telomerase.** *Science* 1994, **266**:404-409.
10. Lingner J, Hughes TR, Shevchenko A, Mann M, Lundblad V, Cech TR: **Reverse transcriptase motifs in the catalytic subunit of telomerase.** *Science* 1997, **276**:561-567.
11. Counter CM, Meyerson M, Eaton EN, Weinberg RA: **The catalytic subunit of yeast telomerase.** *Proc Natl Acad Sci USA* 1997, **94**:9202-9207.
12. 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.
13. Lieber MR, Grawunder U, Wu X, Yaneva M: **Tying loose ends: roles of Ku and DNA-dependent protein kinase in the repair of double-strand breaks.** *Curr Opin Genet Dev* 1997, **7**:99-104.
14. Tsubouchi H, Ogawa H: **A novel *mre11* mutation impairs processing of double-strand breaks of DNA during both mitosis and meiosis.** *Mol Cell Biol* 1998, **18**:260-268.
15. Ivanov EL, Sugawara N, White CI, Fabre F, Haber JE: **Mutations in *XRS2* and *RAD50* delay but do not prevent mating-type switching in *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1994, **14**: 3414-3425.
16. Kleckner N: **Meiosis: how could it work?** *Proc Natl Acad Sci USA* 1996, **93**:8167-8174.
17. Lendvay TS, Morris DK, 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.
18. Barnes G, Rio D: **DNA double-strand-break sensitivity, DNA replication, and cell cycle arrest phenotypes of Ku-deficient *Saccharomyces cerevisiae*.** *Proc Natl Acad Sci USA* 1997, **94**:867-872.
19. Porter SE, Greenwell PW, Ritchie KB, Petes TD: **The DNA-binding protein Hdf1p (a putative Ku homologue) is required for maintaining normal telomere length in *Saccharomyces cerevisiae*.** *Nucleic Acids Res* 1996, **24**:582-585.
20. Kironmai KM, Muniyappa K: **Alteration of telomeric sequences and senescence caused by mutations in *RAD50* of *Saccharomyces cerevisiae*.** *Genes Cells* 1997, **2**:443-455.
21. Wiley EA, Zakian VA: **Extra telomeres, but not internal tracts of telomeric DNA, reduce transcriptional repression at *Saccharomyces* telomeres.** *Genetics* 1995, **139**:67-79.
22. Gravel S, Larrivee M, Labrecque P, Wellinger RJ: **The yeast Ku-complex as a regulator of the chromosomal DNA end-structure.** *Science* 1998, in press.
23. Laroche T, Martin SG, Gotta M, Gorham HC, Pryde FE, Louis EJ, Gasser SM: **Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres.** *Curr Biol* 1998, **8**:653-656.

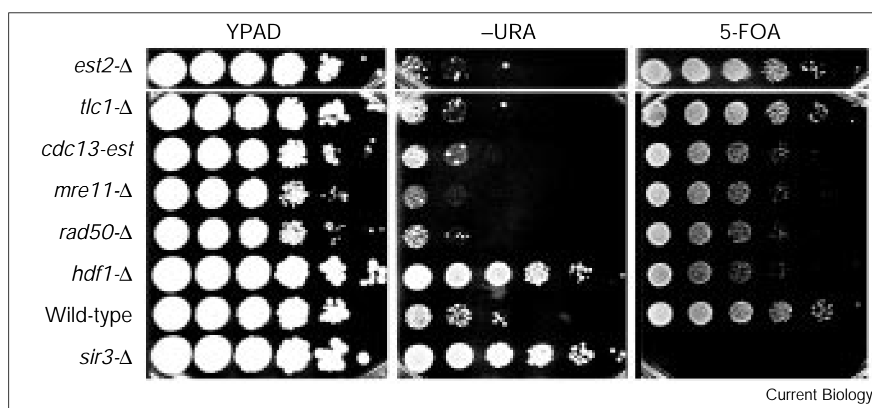
Supplementary material

Telomere maintenance is dependent on activities required for end repair of double-strand breaks

Constance I. Nugent, Giovanni Bosco, Lyle O. Ross, Sara K. Evans, Andrew P. Salinger, J. Kent Moore, James E. Haber and Victoria Lundblad

Current Biology 11 May 1998, **8**:657–660

Figure S1



The Ku heterodimer, but not Cdc13p or telomerase, is required for silencing of telomere-located genes. Serial 10-fold dilutions of cells from freshly grown wild-type UCC3505, *est2-Δ*, *tlc1-Δ*, *cdc13-2^{est}*, *mre11-Δ*, *rad50-Δ*, *hdf1-Δ* and *sir3-Δ* strains were plated on complete media in order to monitor total cell viability, on media lacking uracil to assess the extent of derepression of *URA3* transcription, and on media containing 5-FOA to determine the proportion of cells able to repress *URA3* transcription. Plates were incubated at 30°C for 3 days.

Materials and methods

Yeast strains

Haploid single and double mutant strains were generated by dissection of the relevant heterozygous diploid strain, and genotype was determined via the associated nutritional or drug resistance marker. All strains used in Figures 1 and 2 were derived from a single parental diploid, and thus all haploid strains used in these two figures are isogenic. For the telomeric silencing assays shown in Figure 3, various mutations were introduced directly into the haploid UCC3505 strain [9]. The *est1-Δ1*::*HIS3*, *est2-Δ1*::*URA3* and *tlc1-Δ*::*LEU2* disruptions and the *cdc13-1^{ts}* and *cdc13-2^{est}* alleles have been described previously [7,9,17]. The *HDF1*, *YKU80*, *RAD50* and *MRE11* genes were disrupted using fragments generated from PCR amplification of the kanMX2 cassette [S1]. Primer pairs for each gene were designed with 46 base pairs of homology to regions at the start and stop codons of the open reading frame. The mutant *cdc13-1^{ts}* and *cdc13-2^{est}* alleles of *CDC13* were integrated into UCC3505 by a pop-in/pop-out strategy, using counter-selection against *LYS2* and subsequent determination of the *CDC13* genotype via the presence or absence of a restriction site uniquely associated with each mutation [7]. *SIR3* was disrupted with a fragment containing *sir3-Δ::LEU2* (pLP47, kindly provided by L. Pillus). Additional strains were constructed in strain JKM101. Deletions of *MRE11* and *RAD50* were created as previously described [3] and deletion of *HDF1* was accomplished either as described above, or by *URA3*-marked or *LEU2*-marked deletion of the gene (pBRWS[HDF1] kindly provided by W. Seide, and pSEL1 kindly provided by S. Lee). Double mutants were obtained from heterozygous diploids, as described above. All strains were grown and manipulated at room temperature (approximately 23°C) unless otherwise indicated.

Genetic methods

Mutant screens monitored plasmid-dependent growth phenotypes after loss of pVL438 (*ARS CEN URA3 CDC13*; screen A) or loss of pVL368 (*ARS CEN URA3 EST1*; screen B). Mutagenized colonies were initially plated on –URA media, and colony growth in the absence of the *CDC13* or *EST1* covering plasmid was subsequently monitored after one to two successive replicas to 5-FOA-containing media. All colonies which showed either no growth or reduced growth on the 5-FOA plates were recovered from the –Ura plates and examined for alterations in telomere length. In screen A, 240,000 mutagenized colonies of a *cdc13-1^{ts}*/p*URA3 CDC13* strain were examined for colonies that could not grow or showed reduced growth at 26°C after plasmid loss; candidates were subsequently assayed for alterations in telomere length. A number of strains were identified that exhibited either increases or decreases in telomere length in the presence of the *CDC13* plasmid (data not shown); a subset of mutant isolates with short telomeres are the subject of this report. In screen B, we looked for mutants that were capable of no more than ~10 divisions in an Est$^-$ strain but grew normally if the strain was Est$^+$, using a similar plasmid-dependent screen of an *est1-Δ*/p*URA3 EST1* strain. From 110,000 mutagenized colonies, two mutant strains were recovered that displayed a rapid cell death phenotype in the presence of an *est1-Δ* mutation, and short telomeres in an *EST1* background.

For the growth assays shown in Figures 1–3, serial 10-fold dilutions of haploid strains of the desired genotype were spotted onto appropriate media and incubated at differing temperatures. To ensure that equivalent numbers of cells were compared for each strain, initial cell density was determined using a hemocytometer. A minimum of four isolates of each genotype were tested in these experiments. Yeast transformations, sporulation and tetrad dissections were carried out using standard techniques. Genomic DNA preparations and Southern blots to monitor telomere length were performed as previously described [17].

Reference

S1. Wach A, Brachat A, Pohlmann R, Philippsen P: **New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*.** *Yeast* 1994, **10**:1793-1808.

Table S1

Yeast strains used in this work

Strain	Genotype
DVL 180	Mat a/α *rad50Δ::kanR est1Δ-3::HIS3 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *RAD50 EST1 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
DVL 181	Mat a/α *mre11Δ::kanR est1Δ-3::HIS3 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *MRE11 EST1 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
DVL 188	Mat a/α *yku80Δ::kanR est2Δ-1::URA3 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *yKU80 EST2 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
DVL 209	Mat a/α *hdf1Δ::kanR est2Δ-1::URA3 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *HDF1 EST2 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
DVL 214	Mat a/α *yku80Δ::kanR cdc13-1ts rad9Δ::TRP1 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *yKU80 CDC13 RAD9 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
DVL 215	Mat a/α *hdf1Δ::kanR cdc13-1ts rad9Δ::TRP1 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *HDF1 CDC13 RAD9 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
DVL 187	Mat a/α *mre11Δ::URA3 cdc13-1ts sir3Δ::LEU2 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *MRE11 CDC13 SIR3 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
DVL 229	Mat a/α *yku80Δ::kanR mre11Δ::URA3 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200/CF [ura3::TRP1 SUP11 CEN4 D8B]* *yKU80 MRE11 ura3-52 ade2-101 lys2-801 leu2-Δ1 trp1-Δ1 his3-Δ200*
TVL310	*Mat a tlc1Δ::LEU2 ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ppr1Δ::HIS3 adh4::URA3 (URA3 @ TEL VII_L) DIA5-1 (ADE2 @ TEL V_R)*
TVL312	*Mat a est2Δ::kanR ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ppr1Δ::HIS3 adh4::URA3 (URA3 @ TEL VII_L) DIA5-1 (ADE2 @ TEL V_R)*
TVL314	*Mat a cdc13-2^{est} ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ppr1Δ::HIS3 adh4::URA3 (URA3 @ TEL VII_L) DIA5-1 (ADE2 @ TEL V_R)*
TVL315	*Mat a hdf1Δ::kanR ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ppr1Δ::HIS3 adh4::URA3 (URA3 @ TEL VII_L) DIA5-1 (ADE2 @ TEL V_R)*
TVL316	*Mat a mre11Δ::kanR ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ppr1Δ::HIS3 adh4::URA3 (URA3 @ TEL VII_L) DIA5-1 (ADE2 @ TEL V_R)*
TVL317	*Mat a rad50Δ::kanR ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ppr1Δ::HIS3 adh4::URA3 (URA3 @ TEL VII_L) DIA5-1 (ADE2 @ TEL V_R)*
TVL318	*Mat a sir3Δ::LEU2 ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ppr1Δ::HIS3 adh4::URA3 (URA3 @ TEL VII_L) DIA5-1 (ADE2 @ TEL V_R)*