27. T. A. Cooper, *J. Biol. Chem.* **267**, 5330 (1992).
28. J. D. Dignam, R. M. Lebovitz, R. G. Roeder, *Nucleic Acids. Res.* **5**, 1475 (1983).
29. The sequence specificity of rMBP–CUG-BP binding was increased by a 10-min preincubation in HeLa cell nuclear extracts, which suggests that modification of the protein, most likely phosphorylation, is required for optimal activity.
30. We thank J. Seidman and L. Thierfelder for genomic clones containing hcTNT exon 5; C. Lo for excellent technical assistance; and B. Casey, D. Nelson, and S. Berget for critical comments on the manuscript. Supported by grants from the Muscular Dystrophy Association and the National Institutes of Health (HL45565 to T.A.C. and AR 44387 to L.T.T.).

23 December 1997; accepted 25 March 1998

Yeast Ku as a Regulator of Chromosomal DNA End Structure

Serge Gravel, Michel Larrivée, Pascale Labrecque, Raymund J. Wellinger*

During telomere replication in yeast, chromosome ends acquire an S-phase–specific overhang of the guanosine-rich strand. Here it is shown that in cells lacking Ku, a heterodimeric protein involved in nonhomologous DNA end joining, these overhangs are present throughout the cell cycle. In vivo cross-linking experiments demonstrated that Ku is bound to telomeric DNA. These results show that Ku plays a direct role in establishing a normal DNA end structure on yeast chromosomes, conceivably by functioning as a terminus-binding factor. Because Ku-mediated DNA end joining involving telomeres would result in chromosome instability, our data also suggest that Ku has a distinct function when bound to telomeres.

The Ku protein, comprising two subunits of about 70 and 85 kD, appears to be present in all eukaryotic cells, which suggests that it is involved in a conserved and important function. Originally identified as an autoimmune antigen localized to the nucleus, this protein together with a third component, the catalytic subunit of a DNA-dependent protein kinase (DNA-PK_{cs}), is now known to be critical for nonhomologous DNA double-strand break repair and the site-specific recombination of the V(D)J gene segments (*1*). In vitro, mammalian Ku binds directly to double-stranded (ds) DNA ends in a sequence-independent fashion (*2*). Thus, it has been postulated that Ku associates with dsDNA ends produced by DNA damage or during recombination and may recruit additional factors necessary for successful end joining (*1*).

The yeast *Saccharomyces cerevisiae* contains two genes, *HDF1* and *YKU80/HDF2* (hereafter referred to as *YKU80*), which are homologs of the two mammalian Ku subunits (*3–5*). Like its mammalian counterpart, yeast Ku binds DNA ends only in the heterodimeric form (*5*). Consistent with the proposed roles for Ku in mammalian cells, yeast cells lacking Ku activity are severely impaired in Rad52-independent nonhomologous DNA end joining (NHEJ) (*4–6*). Furthermore, yeast strains devoid of Ku do not grow at elevated temperatures (*3*, *4*) and

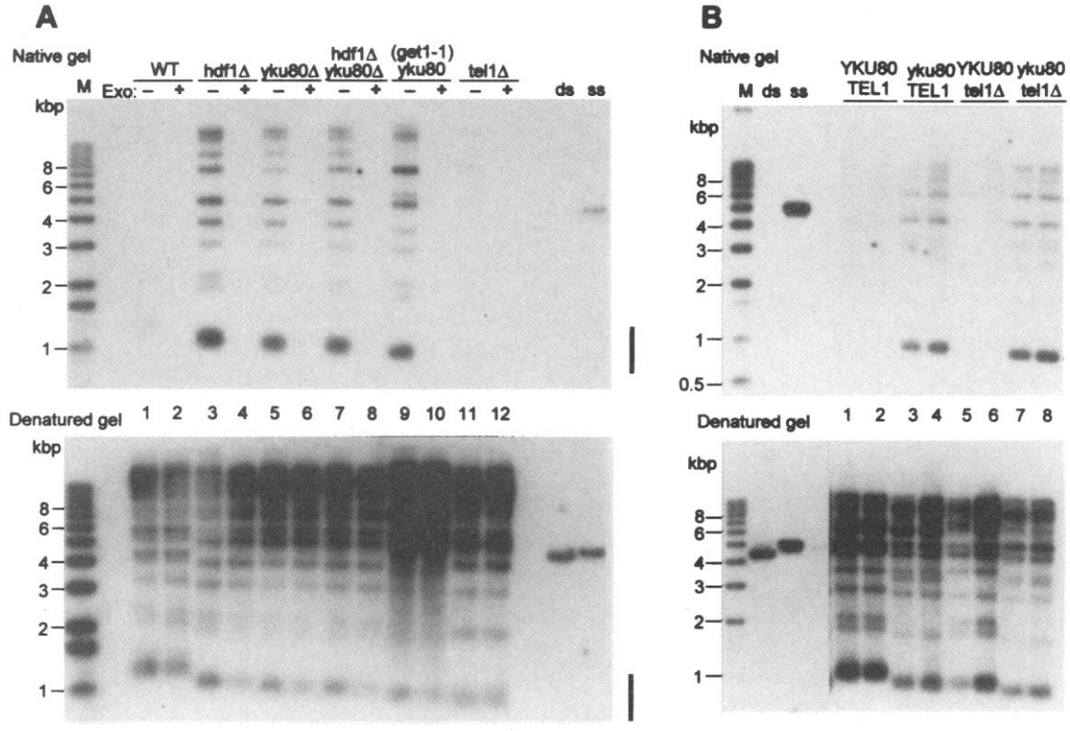

Fig. 1. Altered telomeric end structure caused by mutations in yeast Ku genes. (**A**) (Top) DNA from strains with the indicated genotypes and grown at 23°C was mock treated (lanes 1, 3, 5, 7, 9, and 11) or digested with *E. coli* exonuclease I (lanes 2, 4, 6, 8, 10, and 12). All samples were then digested with Xho I and analyzed by nondenaturing in-gel hybridization with a 22-mer oligonucleotide probe composed of telomeric $C_{1-3}A$ repeats (*12*). About two-thirds of the terminal restriction fragments are of a size indicated by the bar. Additional bands derive from individual telomeres lacking a subtelomeric repeated element Y′. Lanes ss and ds contain DNA with telomeric TG_{1-3} repeats in ss and ds form, respectively. Lane M, end-labeled 1-kb ladder DNA (Gibco-BRL) serving as size standard. (Bottom) Same gel after denaturation of the DNA and rehybridization with the same telomeric probe. The following strains were used: DWY291 (lanes 1 and 2), DWY290 (lanes 3 and 4), DWY292 (lanes 5 and 6), DWY293 (lanes 7 and 8), RWY737d (lanes 9 and 10), KWRY100 (lanes 11 and 12) (*29*). (**B**) A diploid yeast strain formed with haploids KWRY100 (*tel1Δ::HIS3, YKU80*) and RWY739b (*TEL1, yku80*) was sporulated and individual tetrads were dissected. All four spores from a tetratype tetrad were grown at 23°C (lanes 1, 3, 5, and 7) or incubated at 37°C (lanes 2, 4, 6, and 8) before DNA isolation and analysis by nondenaturing hybridization (top), followed by denaturation of the DNA and rehybridization (bottom) as in (A).

harbor dramatically shortened telomeres (*4*, *7*). Recently, Hdf1p has been shown to interact with Sir4p in vivo (*8*). Because Sir4p is a necessary component for transcriptional silencing at several genomic loci, including telomeres, a role for Ku in telomere maintenance has been hypothesized.

The telomeres of virtually all eukaryotic chromosomes are composed of simple direct repeats. Yeast telomeres consist of 250 to 400 base pairs (bp) of $TG_{2-3}(TG)_{1-6}$ repeats (commonly abbreviated TG_{1-3} or $C_{1-3}A$), and the G-rich strand forms the 3′ end of the chromosomes (*9*, *10*). During replication of yeast telomeres, chromosomal ends acquire a transient, single-stranded (ss) extension of the G-rich strand that can be detected in native DNA with telomeric probes (*11*, *12*). In addition, these S-phase–specific overhangs can be detected in cells without telomerase, which suggests that they can be generated and processed by other enzymes (*12*, *13*). Because virtually no overhangs can be detected by hybridization in yeast cells not in S phase (Fig. 1A, lane 1) (*11*, *12*), the terminal DNA configuration in such cells could be blunt or could consist of a short overhang (<20 bases) of either strand.

We reasoned that cells that lack the ability to re-form a normal terminal DNA structure would arrest in late S/G_2 because of an abnormal end structure that might be recognized by DNA damage checkpoints. Thus, a bank of randomly mutagenized and temperature-sensitive (ts) yeast strains was screened for those that arrested in G_2/M at the restrictive temperature; then they were analyzed for their terminal DNA structure by in-gel hybridization to native DNA (*12*). Analysis of an outcrossed strain (RWY737d) that carries a previously uncharacterized mutation is shown (Fig. 1A, lanes 9 and 10) (*14*). In addition to the strong telomeric signals for ss G strands observed in native gels, the overall length of the telomeric repeats was reduced by about 200 bp in this strain (Fig. 1A, bottom; compare lanes 1 and 9).

Three lines of evidence indicate that the mutation in strain RWY737d resides in the *YKU80* gene. First, strains that carry a deletion of the *YKU80* gene did not complement the ts, telomeric length, or DNA end-structure phenotypes, whereas strains that carry a deletion of *HDF1* did complement (*15*). Second, a plasmid expressing a Myc epitope-tagged Yku80 protein complemented all these phenotypes (*16*). Third, when assayed for telomeric repeat length and ss extensions of the G-rich strand, the phenotype of a strain that carries a deletion in *YKU80* was indistinguishable from that of strains that carry the mutation identified here (Fig. 1A, lanes 5 and 6). In addition, strains that carry a deletion of the *HDF1* gene or strains with a deletion of both *HDF1* and *YKU80* genes also displayed the same phenotypes (Fig. 1A, lanes 3, 4, 7, and 8). Digestion of the genomic DNA with *Escherichia coli* exonuclease I, a 3′-specific ssDNA exonuclease (*12*, *17*), eliminated all signals in the native gels (Fig. 1A, top), reduced the hybridization intensity of the terminal restriction fragments after denaturation (Fig. 1A, bottom), and slightly reduced the sizes of these fragments. We estimate that the overhangs are about 50 to 90 bases long (*18*). Thus, the telomeric signals observed for Ku^- cells in native gels correspond to unusually long terminal extensions of the G-rich strand.

For at least some end-joining reactions in mammalian cells, an association of Ku with DNA-PK_{cs} is required (*1*). Yeast encodes a number of genes such as *TEL1* and *MEC1* that share some sequence homologies with the gene for DNA-PK_{cs} (*19*). Most notably, yeast strains with a deletion of *TEL1* also display shortened telomeres (Fig. 1A, bottom, lane 11; Fig. 1B, bottom, lanes 5 and 6) (*20*). However, the terminal DNA in yeast strains with a deletion of *TEL1* had no detectable overhangs (Fig. 1A, top, lanes 11 and 12; Fig. 1B, top, lanes 5 and 6). In addition, strains with a mutation in both *YKU80* and *TEL1* had even shorter telomeric TG_{1-3} tracts than either single mutant and displayed ss extensions (Fig. 1B, lanes 7 and 8) (*7*). Thus, the altered DNA end structure observed for strains with mutations in one of the Ku genes is not simply a consequence of a short telomeric repeat tract but a specific phenotype associated with the lack of Ku. Consistent with this conclusion, the signals for ss G-rich extensions were equally visible whether Ku^- cells were grown at permissive or restrictive temperatures (Fig. 1B, top, lanes 3 and 4). These results also

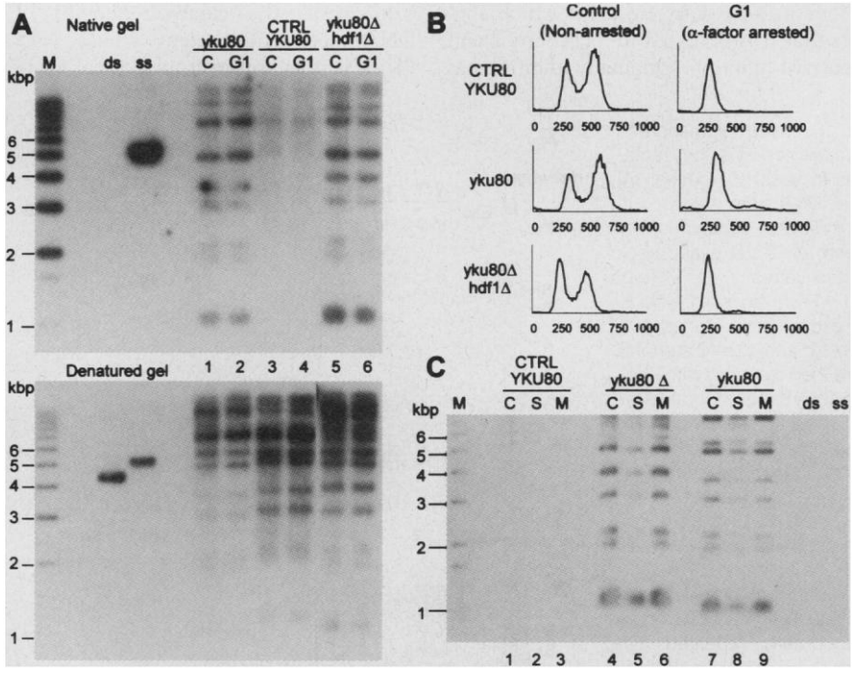

Fig. 2. Analysis of G-strand overhangs throughout the cell cycle. (**A**) Strains RWY738a (*yku80*), RWY738d (*YKU80*), and DWY293 (*hdf1Δ::TRP1*, *yku80Δ::URA3*) were grown to logarithmic phase in rich medium (lanes 1, 3, and 5, marked C) or arrested in G_1 phase by incubation with 0.75 μM α factor for 2 hours (lanes 2, 4, and 6, marked G1). DNA was isolated and analyzed for G-strand overhangs on terminal restriction fragments as in Fig. 1. (**B**) Before DNA isolation, part of the culture analyzed in (A) was stained for DNA with propidium iodide and was analyzed for DNA content by standard flow cytometry. Relative fluorescence is indicated on the *x* axis in arbitrary units. (**C**) Strains DWY291 (*YKU80*), DWY292 (*yku80Δ::URA3*), and RWY737d (*yku80*) were grown to logarithmic phase (lanes 1, 4, and 7, marked C), arrested with 0.4 M hydroxyurea in early S phase (lanes 2, 5, and 8, marked S), or arrested with nocodazole at 20 μg/ml in M phase (lanes 3, 6, and 9, marked M). Flow cytometric DNA analysis showed that >90% of the cells were at the expected arrest points (*23*). Analysis for G-strand overhangs in these samples was as in (A). Denaturation and rehybridization of the gel with the telomeric oligonucleotide showed that all lanes contained about equal amounts of DNA (*23*).

Département de Microbiologie et Infectiologie, Faculté de Médecine, Université de Sherbrooke, 3001 12th Avenue Nord, Sherbrooke, Quebec QC J1H 5N4, Canada.

*To whom correspondence should be addressed. E-mail: R.Wellin@courrier.usherb.ca

support the notion that Ku and Tel1p affect telomere length through different genetic pathways (*7*).

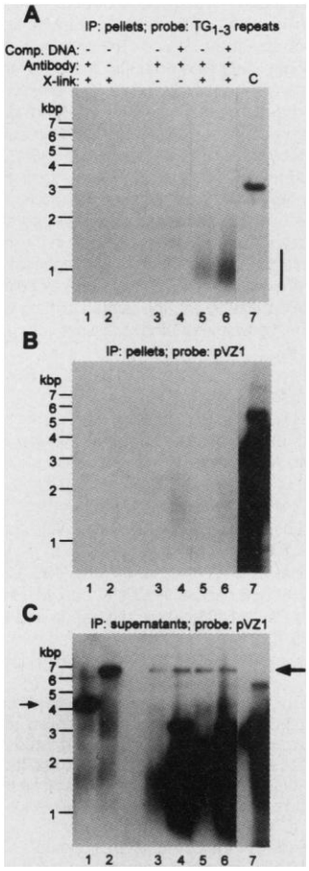

Fig. 3. Immunoprecipitation of telomeric repeat DNA with an antibody specific for a tagged Yku80p after in vivo cross-linking. (**A**) Strain RWY737d (*yku80*) was transformed with pRS316 (lane 1) or with pKU80-myc (lanes 2 to 6) (*16*). Protein-DNA cross-linking, preparation of whole-cell extracts, and immunoprecipitation with the 9E10 antibody were as in (*22*), except that protein G-agarose (Boehringer) was used instead of protein A–Sepharose. DNA from the pellets was deproteinized and analyzed by Southern blotting with a fragment containing 280 bp of TG_{1-3} repeats as probe (*11*). The controls were as follows: lane 1, control plasmid without the YKU80-myc gene; lane 2, no antibodies added; lanes 3 and 4, no cross-linking (X-link) induced. In addition, 0.2 μg of a linearized plasmid containing 280 bp of telomeric repeats (*24*) was added to the cells represented in lanes 4 and 6 after cross-linking (lane 6) or before extract preparation (lane 4). In lane 7, linearized pVZ1 DNA was loaded as a control. Bar indicates the signal for telomeric repeat-containing fragments. IP, immunoprecipitate; Comp. DNA, competitor DNA. (**B**) Blot in (A) was rehybridized to a randomly labeled pVZ1 probe. (**C**) As a control for DNA input, 4% of the DNA that remained in the supernatants and washes after immunoprecipitation was analyzed by Southern blotting with the pVZ1 DNA probe. Thick arrow indicates the linearized pKU80-myc. Small arrow indicates the linearized pRS316 DNA. Heavy smears below 3 kb in lanes 4 and 6 are due to the added competitor DNA.

It was formally possible that a fraction of the Ku^- cell population would delay in late S phase and therefore have telomeres with long overhangs, but for the rest of the cell cycle have normal terminal DNA structures (*21*). To investigate this possibility, we induced *yku80* mutant cells to arrest in G_1, early S, or M phase and then assayed for G-strand overhangs (Fig. 2). Although wild-type strains did not have ss G strands at the telomeres at any of the arrest points, the arrested *yku80* mutant cells always yielded signals that were indistinguishable from those obtained with nonsynchronized mutant cells (Fig. 2, A and C). Thus, whereas the telomeres in wild-type cells acquire G-strand extensions only transiently in late S phase, the telomeres in Ku^- cells have such overhangs throughout the cell cycle.

To determine whether yeast Ku modulates DNA end structures by binding to chromosomal ends, we performed in vivo cross-linking studies. A *yku80* mutant strain was transformed with a plasmid encoding a Myc epitope-tagged Yku80p and protein-DNA cross-links were induced in vivo (Fig. 3) (*22*). After we immunoprecipitated whole cell extracts with the Myc-specific antibody, we analyzed DNA from the pellets and supernatants by Southern blotting. DNA fragments containing telomeric repeats were recovered in immunoprecipitates from the strain harboring the pKU80-myc plasmid (Fig. 3A, lanes 5 and 6) but not from strains lacking Yku80p (Fig. 3A, lane 1). These DNA fragments were not immunoprecipitated in the absence of antibody (Fig. 3A, lane 2) or in the absence of formaldehyde-induced cross-linking (Fig. 3A, lanes 3 and 4). Finally, when a probe specific for the yeast ribosomal DNA repeat was used on the same blot, no signal above background was detectable after immunoprecipitation (*23*).

To verify that the fragments immunoprecipitated were due to in vivo cross-links and not associations that occurred during extract preparation, we added a fivefold excess of a linearized plasmid to the cells before we prepared the extract (*24*). Fragments containing telomeric DNA were immunoprecipitated as efficiently as in the absence of competitor DNA (Fig. 3A, compare lanes 5 and 6), but virtually no competitor DNA was recovered in the pellet (Fig. 3B). These results demonstrate that yeast Yku80p can be cross-linked in vivo to chromosomal DNA composed of telomeric repeats. The exact binding site for Ku on the telomeres

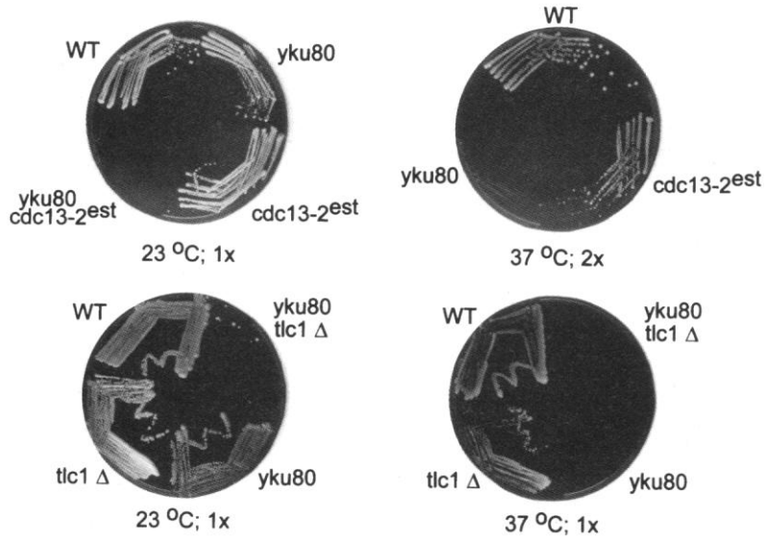

Fig. 4. Accelerated death of cells when a *yku80* mutation is combined with mutations affecting telomerase. (**Top**) Diploid strain RWY80 (*cdc13-2^{est}/CDC13, YKU80/yku80*) (*31*) was sporulated and individual tetrads were dissected. Colonies of a single tetrad were restreaked onto rich medium and incubated at 23°C (left). Cells harboring the *yku80* mutation alone did not grow at 37°C (right) but did not senesce after five successive restreaks at 23°C (*23*). Cells with the *cdc13-2^{est}* mutation were not ts (right), but they ceased to grow after two successive restreaks (*23*). Cells labeled WT (wild type) were not ts and did not show senescence. (**Bottom**) Diploid RWY85 (*tlc1Δ/TLC1, YKU80/yku80*) (*31*) was sporulated, tetrads were dissected, and cell phenotypes were identified as described above. For both crosses, each tetratype tetrad analyzed in this way (six of six) contained one colony from which viable cells could not be recovered and the genetic analysis of the other three colonies indicated that these dead cells were the double mutants. The number of times the cells were restreaked at the indicated temperatures is indicated by 1× and 2×, which correspond to about 30 and 50 generations of growth, respectively.

is unknown, but the in vitro properties of Ku and the altered terminal DNA structure in Ku$^-$ cells strongly suggest that Ku binds at or near the most distal telomeric repeats on yeast chromosomes.

These results show that yeast Ku is involved in establishing the proper terminal DNA structure on yeast chromosomes and that this effect is likely mediated by the direct binding of Ku at or near the chromosome ends. We have also observed a dramatic reduction in the transcriptional repression of *URA3*, a gene required for uracil biosynthesis, when this gene is located near a telomere in Ku$^-$ cells (*25*); this result reinforces the conclusion that Ku is present in normal telomeric chromatin. Surprisingly, Ku$^-$ cells are viable at the permissive temperature, although they have an altered terminal DNA structure. However, yeast cells harboring both a deletion of the *TLC1* gene, which encodes the RNA component of telomerase (*26*), and a *yku80* mutation die after only about 10 generations (Fig. 4). Similar results were obtained when the *cdc13-2^{est}* mutation, which affects telomerase function in vivo (*27*), was combined with a *yku80* mutation (Fig. 4). These results show that defects in components associated with the yeast telomerase are deleterious in strains that do not maintain a normal terminal DNA end structure. It is therefore possible that in rapidly dividing cells grown at elevated temperatures, Ku-mediated establishment of a proper terminal DNA structure would be required for maintaining a functional block of telomeric repeats via telomerase, whereas in cells grown at 23°C normal telomerase activity or alternative recruitment of telomerase by ss G-strand binding proteins would suffice. Alternatively, some aspect of the telomerase-mediated maintenance of telomeric repeats could be inherently sensitive to elevated temperatures.

In addition to its role in DNA end joining (*1*, *5*, *6*), we show here that Ku also binds to chromosome ends, affects the terminal DNA configuration, and is required in the absence of a functional telomerase. However, telomere-to-telomere end-joining reactions would lead to dicentric chromosomes and genomic instability. Therefore, the presence of Ku at yeast telomeres is surprising and suggests that this protein is involved in at least two distinct mechanisms, depending on its binding site. We speculate that interactions of Ku with telomerase-associated proteins or with proteins involved in NHEJ may play a crucial role in distinguishing chromosome ends, where end-to-end fusions are not desirable, from dsDNA breaks within a chromosome. Because the predominant ds break repair mechanism in mammalian cells is NHEJ and because telomeres in these cells are maintained by telomerase, it will be interesting to investigate the roles of Ku in telomere maintenance in such systems.

REFERENCES AND NOTES

1. D. B. Roth, T. Lindahl, M. Gellert, *Curr. Biol.* **5**, 496 (1995); D. T. Weaver, *Trends Genet.* **11**, 388 (1995); M. R. Lieber, U. Grawunder, X. Wu, M. Yaneva, *Curr. Opin. Genet. Dev.* **7**, 99 (1997); S. P. Jackson and P. A. Jeggo, *Trends Biochem. Sci.* **20**, 412 (1995).
2. T. Mimori and J. A. Hardin, *J. Biol. Chem.* **261**, 10375 (1986); S. Paillard and F. Strauss, *Nucleic Acids Res.* **19**, 5619 (1991).
3. H. Feldmann and E.-L. Winnacker, *J. Biol. Chem.* **268**, 12895 (1993); H. Feldmann *et al.*, *ibid.* **271**, 27765 (1996).
4. S. J. Boulton and S. P. Jackson, *Nucleic Acids Res.* **24**, 4639 (1996).
5. G. T. Milne, S. Jin, K. B. Shannon, D. T. Weaver, *Mol. Cell. Biol.* **16**, 4189 (1996).
6. G. J. Mages, H. M. Feldmann, E.-L. Winnacker, *J. Biol. Chem.* **271**, 7910 (1996); W. Siede, A. A. Friedl, I. Dianova, F. Eckardt-Schupp, E. C. Friedberg, *Genetics* **142**, 91 (1996); Y. Tsukamoto, J. Kato, H. Ikeda, *Nucleic Acids Res.* **24**, 2067 (1996); S. J. Boulton and S. P. Jackson, *EMBO J.* **15**, 5093 (1996).
7. S. E. Porter, P. W. Greenwell, K. B. Ritchie, T. D. Petes, *Nucleic Acids Res.* **24**, 582 (1996).
8. Y. Tsukamoto, J. Kato, H. Ikeda, *Nature* **388**, 900 (1997).
9. J. Shampay, J. W. Szostak, E. H. Blackburn, *ibid.* **310**, 154 (1984).
10. E. H. Blackburn and C. W. Greider, Eds., *Telomeres* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 1, 1995); C. W. Greider, *Annu. Rev. Biochem.* **65**, 337 (1996); V. A. Zakian, *Annu. Rev. Genet.* **30**, 141 (1996); R. J. Wellinger and D. Sen, *Eur. J. Cancer* **33**, 735 (1997).
11. R. J. Wellinger, A. J. Wolf, V. A. Zakian, *Cell* **72**, 51 (1993).
12. I. Dionne and R. J. Wellinger, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13902 (1996).
13. R. J. Wellinger, K. Ethier, P. Labrecque, V. A. Zakian, *Cell* **85**, 423 (1996).
14. The Hartwell collection of ts yeast strains (*28*) was analyzed for DNA content, and strains that arrested with a *2n* content were selected for further study. Strains H742 consistently yielded strong telomeric signals in native gels, indicative of abnormally long G-strand extensions. After three successive backcrosses, the ts phenotype associated with strain H742 segregated 2:2 in 13 of 14 tetrads analyzed. Four of these tetrads were analyzed for telomere length and DNA end structure. In all four short telomeres and an overhang of the G-rich strand cosegregated with the ts phenotype. As for wild-type strains, ss C strands were never detectable (*23*). The original designation of the mutation in these strains was *get1-1* (G-strand extensions at telomeres), but once it was realized that *GET1* was allelic to *YKU80/HDF2* (see below), this designation was changed to conform to the currently used gene nomenclature. The precise nature of the *get1-1* mutation is unknown, but because strains harboring this mutation and strains harboring a deletion of the *YKU80* gene display identical phenotypes in all assays used here, we assume that the *get1-1* allele produces a nonfunctional Yku80p. The following individual segregants after the outcrossing were used: RWY738d [*MAT***a**, (*GET1*)*YKU80*], RWY737d and RWY738a [*MAT***a**, (*get1-1*)*yku80*], and RWY739b [*MAT*α, (*get1-1*)*yku80*].
15. Strain RWY737d or RWY739b (*14*) was crossed to strains DWY290, DWY291, DWY292, and DWY293 (*29*) and the indicated phenotypes were tested in diploids (*23*).
16. The *YKU80* gene encoding a COOH-terminal Myc tag was obtained from genomic DNA by polymerase chain reactions (PCRs). This gene contained nucleotides −638 to +1885 with respect to the first ATG of the genomic *YKU80* sequence and 33 nucleotides encoding the 10 amino acids for the Myc tag plus a stop codon at the 3′ end. The protein thus was a full-length, wild-type Yku80p fused to the Myc epitope recognized by the 9E10 monoclonal antibody (Boehringer Mannheim). A 2.5-kb Hind III–Not I fragment containing this gene was then subcloned into the yeast shuttle vector pRS316 (*30*), yielding pKU80-myc. This plasmid, when introduced into strain RWY737d [(*get1-1*)*yku80*], fully complemented the ts, telomeric length, and DNA end-structure phenotypes (*23*) and was also used in the immunoprecipitation studies (Fig. 3). A similar construct has been shown to complement DNA repair deficiencies in *yku80*Δ strains and the tagged protein was also fully functional in end-binding assays with yeast extracts (*5*).
17. I. R. Lehmann and A. L. Nussbaum, *J. Biol. Chem.* **239**, 2628 (1964).
18. The length was estimated from experiments with a variety of restriction enzymes and longer gels (S. Gravel, M. Larrivée, R. J. Wellinger, unpublished data).
19. V. A. Zakian, *Cell* **82**, 685 (1995).
20. A. J. Lustig and T. D. Petes, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 1398 (1986).
21. G. Barnes and D. Rio, *ibid.* **94**, 867 (1997).
22. S. Strahl-Bolsinger, A. Hecht, K. Luo, M. Grunstein, *Genes Dev.* **11**, 83 (1997); V. Orlando and R. Paro, *Cell* **75**, 1187 (1993).
23. S. Gravel, M. Larrivée, R. J. Wellinger, unpublished data.
24. The competitor DNA was a pVZ1 plasmid containing about 280 bp of telomeric repeats derived from plasmid pYLPV (*11*). This plasmid was digested with Not I to yield a linear DNA fragment of 3.5 kb that had a block of 280 bp of telomeric repeats close to one of the ends. pVZ1 is a 3.2-kb bacterial vector derived from pBS + (Stratagene).
25. Repression of the *URA3* gene at telomere VII-L and at *HML*, an internal chromosomal locus normally containing a repressed copy of the α mating-type information, was assessed as in (*26*). When *URA3* is located proximal to a telomere, only 0.1 to 1% of the cells grow on media lacking uracil (*26*). However, in the absence of Ku, about 90% of the cells form colonies on such plates. In contrast, when *URA3* is located at *HML*, transcriptional repression is about equal in wild-type and Ku$^-$ cells (*23*).
26. M. S. Singer and D. E. Gottschling, *Science* **266**, 404 (1994).
27. C. I. Nugent, T. R. Hughes, N. F. Lue, V. Lundblad, *ibid.* **274**, 249 (1996).
28. L. H. Hartwell, *J. Bacteriol.* **93**, 1662 (1967).
29. Strains DWY290 (*hdf1*Δ*::TRP1, YKU80*), DWY291 (*HDF1, YKU80*), DWY292 (*HDF1, yku80*Δ*::URA3*), and DWY293 (*hdf1*Δ*::TRP1, yku80*Δ*::URA3*) were from D. Weaver (*5*). Strain KWRY100 (*MAT***a**, *tel1*Δ*::HIS3*) harbors a complete deletion of the TEL1 coding sequence and was from K. Runge.
30. R. S. Sikorski and P. Hieter, *Genetics* **122**, 19 (1989).
31. Diploids RWY80 and RWY85 were obtained by mating strain RWY739b (*MAT*α, *yku80, CDC13, TLC1*) (*14*) with a strain that contained the *cdc13-2^{est}* mutation (*27*) or with a *tlc1*Δ strain (*26*).

32. We thank E. A. Siewert and M. Winey for analyzing and providing us with the ts mutant strains; D. Weaver, K. Runge, V. Lundblad, and D. Gottschling for supplying yeast strains; K. Runge for insightful suggestions; B. Chabot and K. Runge for input on the manuscript; and V. Lundblad for sharing unpublished data. Supported by grants from the Canadian MRC (MT-12616) and the National Cancer Institute of Canada (007103). R.J.W. is a Chercheur-Boursier Senior of the FRSQ.

28 January 1998; accepted 17 March 1998