Dynamic Regulation of Single-Stranded Telomeres in *Saccharomyces cerevisiae*

Stephanie Smith, Soma Banerjee, Regina Rilo¹ and Kyungjae Myung²

Genome Instability Section, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland 20892

Manuscript received August 24, 2007

Accepted for publication December 12, 2007

ABSTRACT

The temperature-sensitive phenotypes of *yku70Δ* and *yku80Δ* have provided a useful tool for understanding telomere homeostasis. Mutating the helicase domain of the telomerase inhibitor Pif1 resulted in the inactivation of cell cycle checkpoints and the subsequent rescue of temperature sensitivity of the *yku70Δ* strain. The inactivation of Pif1 in *yku70Δ* increased overall telomere length. However, the long G-rich, single-stranded overhangs at the telomeres, which are the major cause of temperature sensitivity, were slightly increased. Interestingly, the rescue of temperature sensitivity in strains having both *pif1-m2* and *yku70Δ* mutations depended on the homologous recombination pathway. Furthermore, the BLM/WRN helicase yeast homolog Sgs1 exacerbated the temperature sensitivity of the *yku70Δ* strain. Therefore, the yKu70-80 heterodimer and telomerase maintain telomere size, and the helicase activity of Pif1 likely also helps to balance the overall size of telomeres and G-rich, single-stranded overhangs in wild-type cells by regulating telomere protein homeostasis. However, the absence of yKu70 may provide other proteins such as those involved in homologous recombination, Sgs1, or Pif1 additional access to G-rich, single-stranded DNA and may determine telomere size, cell cycle checkpoint activation, and, ultimately, temperature sensitivity.

Ku protein was originally identified as an autoantigen recognized by sera from patients with rheumatic disorders (MIMORI and HARDIN 1986; REEVES 1987). Biochemical analyses of the Ku70-Ku80 heterodimer protein demonstrated that it bound in a sequence-nonspecific fashion to virtually all double-stranded DNA ends, including 5'- or 3'-protruding ends, blunt ends (MIMORI and HARDIN 1986), and duplex DNA ending in stem-loop structures (FALZON *et al.* 1993). Ku's DNA-binding activity is believed to function by both holding together broken double-stranded DNA, at least in mammals through its interaction with DNA-PKcs (DOWNS and JACKSON 2004; SPAGNOLO *et al.* 2006), and protecting against end-to-end fusions at telomeres (RIHA *et al.* 2002; JACO *et al.* 2004; MYUNG *et al.* 2004). Inactivation experiments in different species demonstrated that the Ku70-Ku80 heterodimer is important for the protection of DNA ends during both DNA repair and telomere maintenance (NUSSENZWEIG *et al.* 1996; GU *et al.* 1997; OUYANG *et al.* 1997; BOULTON and JACKSON 1998; NUGENT *et al.* 1998; BAILEY *et al.* 1999; HSU

et al. 1999; SAMPER *et al.* 2000; FRIESLAND *et al.* 2003; DOWNS and JACKSON 2004; JACO *et al.* 2004; MYUNG *et al.* 2004).

Telomeres are specific DNA structures at the ends of chromosomes that secure genetic information by protecting chromosomes from degradation with the help of many telomere-associated proteins (LINGNER and CECHE 1998; DE LANGE 2002). In *Saccharomyces cerevisiae*, telomeric DNA is 250–400 bp long with a simple repeat tract, C₁₋₃A/TG₁₋₃ (VEGA *et al.* 2003). A G-rich, single-stranded tail is generated at the ends of the telomeres in late S-phase, presumably by the nuclease activity (WELLINGER *et al.* 1993; MARINGELE and LYDALL 2002; BERTUCH and LUNDBLAD 2004). In wild-type cells, telomerase extends the G-rich strand followed by general DNA replication that fills in the opposite C-strand and, therefore, the G-rich, single-stranded tail is not detected in other phases of the cell cycle. Telomere length is affected by many factors, including DNA replication, telomere synthesis by telomerase, and the level of degradation protection provided by telomere maintenance proteins (LINGNER and CECHE 1998; DE LANGE 2002; VEGA *et al.* 2003). Mutations in *yKU70* or *yKU80* decreased overall telomere length and led to an elongation of G-rich, single-stranded tails in all cell cycles (PORTER *et al.* 1996; TSUKAMOTO *et al.* 1997; BOULTON and JACKSON 1998; NUGENT *et al.* 1998). In addition to the perturbation of telomeric structures, the

¹Present address: University of California at Davis, Sacramento, CA 95616.

²Corresponding author: Genome Instability Section, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, Bldg. 49, Room 4A22, Bethesda, MD 20892. E-mail: kmyung@nhgri.nih.gov

mutation of *yKU70* or *yKU80* altered telomere position effect, which was defined as a suppression of gene expression in a subtelomeric region (BOULTON and JACKSON 1998; EVANS *et al.* 1998; LAROCHE *et al.* 1998; NUGENT *et al.* 1998).

yku70Δ or *yku80Δ* mutations cause a growth defect at 37° (FELDMANN and WINNACKER 1993; BOULTON and JACKSON 1996; BARNES and RIO 1997). This temperature sensitivity is the result of telomere homeostasis defects that activate cell cycle checkpoints, and not the result of deficiencies in DNA double-strand break repair pathways, such as nonhomologous end joining (NHEJ) (NUGENT *et al.* 1998; TEO and JACKSON 2001; LEWIS *et al.* 2002; MARINGELE and LYDALL 2002). In these studies, the temperature sensitivity of *yku70Δ* or *yku80Δ* strains was rescued either by mutations in checkpoint genes or in *EXO1* or by the overexpression of telomerase subunits, including *EST2*, *TLC1*, or *EST1*. Interestingly, the overexpression of telomerase subunits did not change telomere length or the G-rich, single-stranded overhangs at telomeres, despite the suppression of cell cycle checkpoint activation elicited by the *yku* mutation at 37° (NUGENT *et al.* 1998; TEO and JACKSON 2001; LEWIS *et al.* 2002). How cell cycle checkpoints are repressed by telomerase overexpression remains unclear.

Mutations in yeast *S. cerevisiae* *Pif1*, which encodes a DNA helicase, were identified in a screen to detect telomere maintenance proteins (SCHULZ and ZAKIAN 1994). Yeast *Pif1* encodes a transcript that makes two alternatively translated proteins controlled by two distinct initiating methionine codons (LAHAYE *et al.* 1991; SCHULZ and ZAKIAN 1994). Both the nuclear and mitochondrial Pif1 proteins have a 5'-3' DNA helicase activity (LAHAYE *et al.* 1991; SCHULZ and ZAKIAN 1994). The *pif1-m2* allele, which inactivates only the nuclear function of Pif1, results in elongated telomeres, the addition of telomeres at telomere seed sequences placed at subtelomeric sites, and an increased rate of spontaneous gross chromosomal rearrangements (SCHULZ and ZAKIAN 1994; ZHOU *et al.* 2000; MYUNG *et al.* 2001a). On the other hand, the *pif1-m1* allele, which abrogates the mitochondrial Pif1, results in a petite phenotype.

The temperature-sensitive phenotype of *yku70Δ* has been a useful tool for determining which proteins are required for the maintenance of telomeric structures. In this study, we found that the temperature-sensitive phenotype of *yku70Δ* was suppressed by the mutation of the telomerase inhibitor Pif1. The suppression of the temperature-sensitive phenotype of *yku70Δ* was due to the active role of homologous recombination (HR)-dependent end protection.

MATERIALS AND METHODS

General genetic methods: Media for propagating yeast strains used in this study are yeast extract–peptone–dextrose

(YPD) media containing 1% (w/v) yeast extract, 2% (w/v) peptone, and 2% (w/v) dextrose, synthetic drop-out (SD) media containing 0.67% (w/v) yeast nitrogen base without amino acids, and 2% (w/v) dextrose with appropriate amino acids depending on the strains used. To make solid media, 1.5% (w/v) agar for YPD plates and 2.3% (w/v) for SD plates were added. All *S. cerevisiae* strains were propagated at 25°, 30°, or 37° as indicated. All *S. cerevisiae* strains used in this study were derived from the S288c parental strain RDY3023 [*MATa*, *ura3-52*, *leu2Δ1*, *trp1Δ63*, *his3Δ200*, *lys2ΔBgl*, *hom3-10*, *ade2Δ1*, *ade8*] and detail genotypes or strains used in this study are listed in Table 1. Strains used in this study were generated by standard polymerase chain reaction (PCR)-based gene-disruption methods, and correct gene disruptions were verified by PCR as described (MYUNG *et al.* 2001c). The sequences of primers used to generate disruption cassettes and confirm disruption of indicated genes are available upon request. Yeast transformation, yeast chromosomal DNA isolation, and PCR were performed as previously described (MYUNG *et al.* 2001c; SMITH *et al.* 2004). The overexpression of the *RAD52* gene was achieved with a multicopy *Yep13* plasmid carrying the *RAD52* open reading frame with 4 kb upstream of the translation initiation codon.

Determination of length of telomeres and G-rich, single-stranded DNA at telomeres: Telomere lengths were determined by *XhoI* digestion of chromosomal DNA, followed by size fractionation and Southern hybridization with a TG repeat probe as described (BANERJEE and MYUNG 2004). The amount of single-stranded DNA at telomeres was determined by a comparison of telomeric signals by Southern hybridization with a TG repeat probe in nondenaturing in-gel hybridization with signals from denaturing conditions.

Northern hybridization: RNA was prepared by using Gentra Systems Purescript RNA purification system from exponential cultures grown for an additional 4 hr at either 30° or 37° after a 1:50 dilution of overnight cultured yeast at 30°. RNA samples were boiled in the presence of ethidium bromide and formaldehyde, cooled, and then loaded onto a 1.2% agarose, 1× MOPS, 1% formaldehyde gel in 1× RNA gel loading dye. Electrophoresis was performed at 100 V for 1 hr. The RNA was transferred to a nitrocellulose membrane and crosslinked with UV. Prehybridization was carried out for 2 hr at 68° in 0.5 M sodium phosphate, 7% (w/v) SDS, 1 mM EDTA (pH 7.0), followed by hybridization with radiolabeled probe. DNA for making a probe to detect *HUG1* expression was generated by PCR with the primers PRKJM 1107 (5'-GACCATGGAC CAAGGCTTAACCCAAAG-3') and PRKJM1108 (5'-CAGAA AGACCGCCGCGACGTTCCGACGCG-3'). The DNA for a control probe to detect *ACT1* expression was made by PCR with the primers PRKJM959 (5'-CTCAATCCAAGAGAGGTATCTT GAC-3') and PRKJM960 (5'-GTGGTGGAGAAAGAGTAACCA CGTTC-3'). Both PCR products were then radiolabeled with [α -³²P]dCTP by random priming as previously described (BANERJEE and MYUNG 2004).

Western blotting: Cell extracts were prepared from exponential cultures grown for an additional 4 hr at either 30° or 37° after 1:50 dilution of overnight cultured yeast at 30°. Cultures were harvested and washed with 20% trichloroacetic acid, glass beads were used to break the cell walls, and the collected cells were resuspended in 1× SDS loading buffer and 2 M Tris. Samples were boiled and centrifuged before loading onto a 7–12% SDS-PAGE (Bio-Rad, Hercules, CA). The protein was transferred to a PVDF membrane. The membranes were blocked in 1× Western blocking reagent (Roche) and then incubated with an anti-Rad53 antibody (Santa Cruz). After washing and incubation in the secondary anti-goat HRP antibody, detection was achieved using Western blocking detection reagents (GE Healthcare).

TABLE 1
***S. cerevisiae* strains used in this study**

Strains	Relevant genotype	Source
RDKY3023	Wild type	CHEN and KOLODNER (1999)
RDKY3615	Wild type, RDKY3023 + <i>hxt13::URA3</i>	CHEN and KOLODNER (1999)
RDKY3633	RDKY3615 + <i>mre11::HIS3</i>	CHEN and KOLODNER (1999)
RDKY3636	RDKY3615 + <i>rad51::HIS3</i>	CHEN and KOLODNER (1999)
RDKY3638	RDKY3615 + <i>rad57::HIS3</i>	CHEN and KOLODNER (1999)
RDKY4343	RDKY3615 + <i>pif1-m2</i>	MYUNG <i>et al.</i> (2001a)
RDKY4393	RDKY3615 + <i>pif1-m1</i>	MYUNG <i>et al.</i> (2001a)
RDKY4399	RDKY3615 + <i>pif1::KAN</i>	MYUNG <i>et al.</i> (2001a)
RDKY4421	RDKY3615 + <i>rad52::HIS3</i>	MYUNG <i>et al.</i> (2001a)
RDKY4423	RDKY3615 + <i>rad59::TRP1</i>	MYUNG <i>et al.</i> (2001a)
RDKY4425	RDKY3615 + <i>rdh54::TRP1</i>	MYUNG <i>et al.</i> (2001a)
YKJM245	RDKY3615 + <i>yku70::TRP1</i>	This study
YKJM1589	RDKY3615 + <i>yku70::HIS3</i>	This study
YKJM1763	RDKY3615 + <i>pif1-m2, yku70::HIS3, rad52::TRP1</i>	This study
YKJM2042	RDKY3023 + <i>pif1-K264A</i>	This study
YKJM2056	RDKY3023 + <i>pif1-K264A, yku70::TRP1</i>	This study
YKJM2133	RDKY3615 + <i>rad54::TRP1</i>	This study
YKJM2189	RDKY3615 + <i>pif1-m2, yku70::HIS3</i> + pRS314	This study
YKJM2191	RDKY3615 + <i>pif1-m2, yku70::HIS3</i> + pRS314-yPIF1	This study
YKJM2381	RDKY3615 + <i>pif1-m2, yku70::HIS3, lif1::TRP1</i>	This study
YKJM2402	RDKY3615 + <i>pif1-m2, yku70::HIS3, rad51::TRP1</i>	This study
YKJM2404	RDKY3615 + <i>pif1-m2, yku70::HIS3, rad54::TRP1</i>	This study
YKJM2406	RDKY3615 + <i>pif1-m2, yku70::HIS3, rad57::TRP1</i>	This study
YKJM2408	RDKY3615 + <i>pif1-m2, yku70::HIS3, rad59::TRP1</i>	This study
YKJM2410	RDKY3615 + <i>pif1-m2, yku70::HIS3, rdh54::TRP1</i>	This study
YKJM2544	RDKY3615 + <i>yku70::TRP1</i> + Yep13-RAD52	This study
YKJM2548	RDKY3615 + <i>yku70::TRP1</i> + Yep13	This study
YKJM2503	RDKY3615 + <i>pif1-m2, yku70::HIS3, mre11::TRP1</i>	This study
YKJM2598	RDKY3615 + <i>pif1-m2, yku70::HIS3, nej1::TRP</i>	This study
YKJM2777	RDKY3615 + <i>pif1-m2, lig4::HIS3, yku70::TRP1</i>	This study
YKJM3303	RDKY3023 + <i>pif1-m2, yku70::TRP1</i>	This study
YKJM3955	RDKY3023 + <i>pif1-m2, cdc13-1</i>	This study
YKJM3957	RDKY3023 + <i>cdc13-1</i>	This study

All strains are isogenic with the wild-type strains, RDKY3023 [*ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8*], or RDKY3615 [*ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8, hxt13::URA3*] that are derived from the Winston 288c background with the exception of the indicated mutations followed by a plus sign.

RESULTS

***pif1* mutations rescued the temperature sensitivity of *yku70Δ* and *cdc13-1* strains:** We recently observed that the overexpression of yKu70-yKu80 in the *pif1-m2* strain caused cell cycle arrest at S-phase in a cell cycle checkpoint-dependent manner (BANERJEE *et al.* 2006). Similarly, in the same study, we showed that the overexpression of Pif1, in either *yku70Δ* or *yku80Δ* strains, also caused growth arrest. Since the overexpression of Pif1 in *yku70Δ* generated a growth defect, we hypothesized that the inactivation of Pif1 could rescue the growth defect of *yku70Δ* at 37°. Wild-type, *yku70Δ*, *pif1-m2* (which inactivates only nuclear Pif1), and *pif1-m2 yku70Δ* strains growing exponentially at 30° were serially diluted and spotted onto two YPD plates. One plate was incubated at 30° and the other at 37°. Similar to previous observations (FELDMANN and WINNACKER 1993; BOULTON and JACKSON 1996; BARNES and RIO 1997),

the *yku70Δ* strain showed a growth defect at 37°, unlike wild type and *pif1-m2* (Figure 1A).

In accordance with our hypothesis, the *pif1-m2* mutation rescued the temperature-sensitive phenotype of *yku70Δ*. A similar rescue of growth defect occurred when the entire *PIF1* gene was completely deleted in *yku70Δ* (Figure 1A). However, the *pif1-m1* allele, which is defective only in mitochondrial Pif1, did not rescue the temperature-sensitive phenotype of *yku70Δ*. The K264A mutation, which disrupts Pif1's helicase activity, also rescued the temperature sensitivity of *yku70Δ* (Figure 1A), suggesting that the helicase activity of Pif1 is necessary for the temperature-sensitive phenotype of the *yku70Δ* strain. The expression of Pif1 in a single-copy plasmid under its own promoter in the *pif1-m2 yku70Δ* strain restored temperature sensitivity at 37° (Figure 1B).

The *cdc13-1* strain has a mutation in the region of Cdc13, which interacts with telomerase and therefore generates large, G-rich, single-stranded overhangs sim-

FIGURE 1.—The temperature-sensitive phenotype of *yku70Δ* is rescued by *pif1* mutations. (A) *pif1* mutations, including *pif1-m2* (nuclear Pif1 defective), *pif1Δ* (complete deletion), and *pif1K264A* (ATPase and helicase defective), rescued the temperature sensitivity of *yku70Δ*, whereas the *pif1-m1* mutation (mitochondrial Pif1 defective) could not. *pif1K264A* and *pif1Δ* exhibit a petite phenotype due to mitochondrial defects that caused these cells to grow more slowly compared to other clones. (B) The expression of full-length Pif1 in *pif1-m2 yku70Δ* restored the temperature-sensitive phenotype. (C) The temperature-sensitive phenotype of *cdc13-1* is partially rescued by the *pif1-m2* mutation.

ilar to the *yku70Δ* strain (WEINERT and HARTWELL 1993; MARINGELE and LYDALL 2002). The *cdc13-1* strain also exhibits temperature sensitivity at 30° (Figure 1C). The *pif1-m2* mutation partially suppressed the temperature sensitivity of the *cdc13-1* strain at 30°, but not at 37°, similar to what has been previously reported (Figure 1C) (DOWNEY *et al.* 2006). Therefore, although perhaps not exactly identical, the mechanism of temperature sensitivity suppression by the *pif1* mutation appears to be similar in *yku70Δ* and *cdc13-1*.

The length of telomeres and the amount of G-rich, single-stranded overhangs are increased by the *pif1-m2* mutation in *yku70Δ*: Telomere size is increased by *pif1* mutations (SCHULZ and ZAKIAN 1994; ZHOU *et al.* 2000). We speculated that *pif1* mutations would increase the telomere size of the *yku70Δ* strain to wild-type levels. When we compared the telomere sizes of wild-type, *yku70Δ*, *pif1-m2*, and *pif1-m2 yku70Δ*, we found that an

additional *pif1-m2* mutation in *yku70Δ* did increase overall telomere length but not to that of wild type at either 30° or 37° (Figure 2A and data not shown). Four independent clones carrying *pif1-m2 yku70Δ* mutations all showed an increase in telomere length compared to the *yku70Δ* strain, similar to what has been recently observed in *pif1Δ yku70Δ* strains (VEGA *et al.* 2007). Mutation of *yKU70* also increases the length of G-rich, single-stranded overhangs at telomeres in all phases of the cell cycle (POLOTNIANKA *et al.* 1998). It has been suggested that the G-rich, single-stranded overhang in *yku70Δ* is a major signal for the activation of cell cycle checkpoints and ultimately temperature sensitivity (MARINGELE and LYDALL 2002). However, we did not find any significant decrease of G-rich, single-stranded overhangs by the *pif1-m2* mutation in *yku70Δ* measured by native in-gel hybridization (Figure 2B). We did observe even a slight increase in the amount of G-rich, single-stranded overhangs in two independent *pif1-m2 yku70Δ* clones and concluded that the inactivation of the Pif1 helicase increased the length of telomeres and the G-rich, single-stranded overhangs.

The overexpression of telomerase subunits suppressed the activation of cell cycle checkpoints although it did not change the length of telomeres or G-rich, single-stranded overhangs (NUGENT *et al.* 1998; TEO and JACKSON 2001; LEWIS *et al.* 2002). To determine whether the *pif1-m2* mutation also suppresses cell cycle checkpoint activation in *yku70Δ*, the expression of *HUG1* was monitored when cells were cultured at 37°. *HUG1* is a small open reading frame whose expression is induced by different kinds of DNA damage in a cell cycle checkpoint-dependent manner (BASRAI *et al.* 1999). Whereas the expression of *HUG1* was induced when *yku70Δ* cells were shifted to 37°, the *pif1-m2 yku70Δ* strain displayed no induction of *HUG1* expression (Figure 2C). Rad53 phosphorylation, another indication of cell cycle checkpoint activation, was not observed in *pif1-m2 yku70Δ*, unlike *yku70Δ*, at 37° (Figure 2D). Therefore, both the overexpression of telomerase subunits (NUGENT *et al.* 1998; TEO and JACKSON 2001; LEWIS *et al.* 2002) and the inactivation of Pif1 rescued the temperature-sensitive phenotype of *yku70Δ* and suppressed the activation of cell cycle checkpoints.

The rescue of *yku70Δ* temperature sensitivity by the inactivation of Pif1 requires HR proteins: The rescue of the temperature sensitivity of *yku70Δ* by the *exo1Δ* mutation suggested that the production of G-rich, single-stranded overhangs at telomeres by Exo1 in the absence of Ku is essential for both checkpoint activation and temperature sensitivity (MARINGELE and LYDALL 2002). Therefore, it is possible that these overhangs could be amended to prohibit cell cycle checkpoint activation in *pif1-m2 yku70Δ* without significantly altering the length of telomeres.

There are at least two distinct DNA repair mechanisms for the alteration of DNA structures: HR and

FIGURE 2.—Cell cycle checkpoint activation is abrogated by the *pif1-m2* mutation in *yku70Δ* at 37°. (A) Telomere sizes from wild-type, *yku70Δ*, *pif1-m2*, *pif1-m2 yku70Δ*, and *pif1-m2 yku70Δ rad52Δ* strains were compared. (B) Chromosomal DNAs from wild-type, *yku70Δ*, *pif1-m2*, and *pif1-m2 yku70Δ* strains were hybridized with telomeric probe by a native in-gel hybridization to detect G-rich, single-stranded overhangs at telomeres. (C) The activation of cell cycle checkpoints in *yku70Δ* at 37° demonstrated by the *HUG1* mRNA expression disappeared by the *pif1-m2* mutation. (D) The phosphorylation of Rad53 in *yku70Δ* at 37° was abrogated by the *pif1-m2* mutation. MMS-treated yeast cell extract (lane 2) was used to demonstrate Rad53 phosphorylation as a positive control.

NHEJ (AYLON and KUPIEC 2004; KROGH and SYMINGTON 2004). To determine whether these repair machineries are responsible for the temperature sensitivity rescue of *yku70Δ* by the inactivation of Pif1, genes responsible for HR were deleted in the *pif1-m2 yku70Δ* strain. Interestingly, the *rad51Δ pif1-m2 yku70Δ* triple mutant resulted in the restoration of a temperature-sensitive phenotype at 37° (Figure 3A). Similar results were observed when a *rad52Δ*, *mre11Δ*, or *rad54Δ* mutation was combined with the *pif1-m2 yku70Δ* strain (Figure 3A; supplemental Figure 1 at <http://www.genetics.org/supplemental/>). Mutating *RAD51*, *RAD52*, *MRE11*, or *RAD54* in the *pif1-m2* strain did not result in temperature sensitivity at 37° (data not shown). The *mre11Δ* mutation in the *pif1-m2 yku70Δ* strain was slightly growth retarded even at 30° (Figure 3A), consistent with previous results that mutations in both *mre11Δ* and *yku70Δ* cause growth defects and severe temperature sensitivity (MARINGELE and LYDALL 2002). Furthermore, the overexpression of Rad52 in the *yku70Δ* strain rescued the temperature sensitivity of *yku70Δ* (Figure 3B). However, the *rad57Δ*, *rad59Δ*, and *rdh54Δ* mutations did not affect the temperature-resistant phenotype of *pif1-m2 yku70Δ* (supplemental Figure 1). In contrast to HR,

mutations of NHEJ genes, including *LIG4*, *LIF1*, or *NEJ1*, did not affect the temperature-resistant phenotype of *pif1-m2 yku70Δ* (Figure 4).

Although mutation of *RAD52* in the *pif1-m2 yku70Δ* strain restored temperature sensitivity, we found no detectable alteration in telomere length (Figure 2A). These data suggest that, while telomere length restoration may contribute, HR events are required for the temperature-resistant phenotype of *pif1-m2 yku70Δ*.

An additional *sgs1* mutation enhanced temperature sensitivity in the *yku70Δ* strain as well as in the *pif1-m2 yku70Δ* strain (Figure 5). Sgs1 is a yeast homolog of human WRN and BLM helicases (GANGLOFF *et al.* 1994; WATT *et al.* 1995). Therefore, in addition to HR proteins, Sgs1 also functions to prevent cell cycle checkpoint activation in *yku70Δ*.

DISCUSSION

The helicase activity of Pif1 has been suggested to block the interaction between telomeric DNA and the RNA subunit of telomerase (BOULE *et al.* 2005). In addition to directly inhibiting telomerase, Pif1 also has a role in general DNA replication. Pif1 is required for

FIGURE 3.—HR proteins actively engage at telomeres to stabilize telomeric structure to rescue temperature sensitivity in the *pif1-m2 yku70Δ* strain. (A) Mutations of HR genes, including *RAD51*, *RAD52*, and *MRE11*, made *pif1-m2 yku70Δ* sensitive to high temperature. (B) The overexpression of Rad52 partially suppressed the temperature-sensitive phenotype of *yku70Δ*.

replication fork pausing at the Fob1-dependent replication fork barrier in the rDNA repeat array (IVESSA *et al.* 2000) and could promote the formation of the long flap structure during RNA removal by Dna2 on the basis of the observation of the rescue of the lethal phenotype of the *DNA2* deletion by the *pif1* mutation (BUDD *et al.* 2006). The rescue of the temperature-sensitive phenotype of the *yku70Δ* strain could be caused by the absence of any one of Pif1's functions. The temperature sensitivity in the absence of the Ku proteins seems to be largely due to defects in telomere maintenance, since temperature sensitivity could be partially suppressed by the overexpression of telomerase subunits (NUGENT *et al.* 1998; TEO and JACKSON 2001; LEWIS *et al.* 2002) and an additional mutation in any telomerase subunit in the *yku70* strain caused an almost synthetic lethal phenotype (GRAVEL *et al.* 1998; NUGENT *et al.* 1998; POLOTNIANKA *et al.* 1998). The *pif1-m2* mutation also rescued the temperature-sensitive phenotype of *cdc13-1*, which also exhibits telomere defects (Figure 1C) (WEINERT and HARTWELL 1993; MARINGELE and LYDALL 2002). Therefore, the rescue of the temperature-sensitive phenotype by the *pif1-m2* mutation in *yku70Δ* is likely due to the restoration of telomere functions. However, we could not exclude completely the possibility that the inactivation of the general DNA replication function of

FIGURE 4.—Nonhomologous end joining proteins, Lig4, Lif1, and Nej1 are not required to rescue the temperature sensitivity in the *pif1-m2 yku70Δ* strain.

Pif1 could indirectly lead to the rescue of temperature sensitivity of *yku70Δ*.

The absence of Ku hinders the recruitment of telomerase to telomeres completely in G₁-phase and significantly in late S-phase (FISHER *et al.* 2004). The inactivation of Pif1 consequently could allow telomerase to be enriched at telomeres. Neither Pif1 inactivation by the *pif1-m2* mutation (Figure 2A) (VEGA *et al.* 2007) nor the overexpression of telomerase subunits, which also rescues the temperature sensitivity of *yku70Δ* (NUGENT *et al.* 1998; TEO and JACKSON 2001; LEWIS *et al.* 2002), was able to restore the length of telomere or C-strand synthesis to a wild-type level. Therefore, any enrichment of telomerase does not appear to restore DNA replication at telomeres. In the absence of Pif1, single-stranded overhangs might form a stable structure and block the activation of cell cycle checkpoints by HR proteins (Figure 6). Although we do not know the exact structure stabilized by HR proteins, this structure is not what has been observed in survivors that display substantial recombination-dependent amplification of telomeres because the *pif1* mutation did not change telomere size similar to those observed in survivors (Figure 2A). We speculate that HR proteins promote the generation of stable G-strand loops similar to the t-loop observed in mammals.

During the review of our manuscript, a study was published suggesting that the *pif1Δ* mutation rescued

FIGURE 5.—The *sgs1Δ* mutation enhanced temperature sensitivity in *yku70Δ* and *pif1-m2 yku70Δ*.

the temperature sensitivity of the *yku70Δ* strain by increasing telomere size (VEGA *et al.* 2007). Similarly, we also observed an increase of telomere size by the *pif1-m2* mutation (Figure 2A). However, because the addition of the *rad52Δ* mutation in the *pif1-m2 yku70Δ* strain restored the temperature-sensitive phenotype (Figure 3) but did not further increase or decrease telomere size (Figure 2A), it is likely that the observed increase of telomere size by the *pif1-m2* mutation is not the sole factor determining temperature resistance.

The stabilization of telomeric structures by HR proteins in *yku70* requires Rad51, Rad52, Rad54, and Mre11 but not Rad55, Rad57, Rdh54, and Rad59 (Figure 3A; supplemental Figure 1 at <http://www.genetics.org/supplemental/>). The Rad55–Rad57 complex is required to stabilize Rad51 filament formation and also to make Rad51 coated single-stranded DNA homologous pairing (SUGAWARA *et al.* 2003). It is possible that this activity might not be required at telomeres or that Rad55–Rad57 may not be required for HR at 37° since the radiation sensitivity and recombination-defect phenotypes of *rad55Δ* and *rad57Δ* were observed only at cold temperatures (LOVETT and MORTIMER 1987). Rad59 and Rdh54 have roles in Rad51-independent recombination (KROGH and SYMINGTON 2004) and are presumably not required for the stabilization of the G-rich, single-stranded DNA at telomeres in the absence of yKu and Pif1.

The *sgs1* mutation aggravated temperature sensitivity of *yku70Δ* and *pif1-m2 yku70Δ* (Figure 5). Sgs1 and its human homolog, the BLM helicase, unwind different DNA structures. Hyperrecombination phenotypes such as increase of sister-chromatid exchange rate or heteroallelic recombination in mitotic cells caused by mutations in these genes suggest that Sgs1 and HR proteins operate in the same HR repair pathway (ONODA *et al.*

FIGURE 6.—A model for how telomeric structures are dynamically regulated.

2000; MYUNG *et al.* 2001b; ONODA *et al.* 2001; HICKSON 2003). The aggravating phenotype by the *sgs1* mutation in the *pif1-m2 yku70Δ* strain could be explained by the lack of HR repair pathway for temperature resistance.

Sgs1 and BLM can unwind G-quadruplex structures (SUN *et al.* 1999; HAN *et al.* 2000; LI *et al.* 2001; HUBER *et al.* 2002). Intriguingly, the *pif1-m2 yku70Δ* strain became sensitive to high temperature in the presence of *N*-methyl mesoporphyrin IX that specifically binds to the G-quadruplex structure and blocks its unwinding (supplemental Figure 2 at <http://www.genetics.org/supplemental/>) (HAN *et al.* 2000; LI *et al.* 2001; WU and MAIZELS 2001; JOYCE and MCGOWN 2004). At high temperatures, the G-rich, single-stranded overhangs could form a new DNA structure and cause lethality in a *yku70Δ* background. It has been proposed that G-quadruplex structures form at telomeres under certain conditions (WILLIAMSON *et al.* 1989; ZAHLE *et al.* 1991). Therefore, if Ku proteins are absent, G-rich, single-stranded overhangs are modified to structures, which not only are sensitive to NMM at 37° but also are unwound by Sgs1. The persistence of this structure might be the cause of cell cycle checkpoint activation. The removal of Pif1 would then hypothetically allow the recruitment of HR proteins, forming another more stable telomeric structure.

Both Sgs1 and Pif1 encode helicases functioning at telomeres, at least in certain conditions. Although the substrate specificities for these helicases are different, there is a possibility that Sgs1 and Pif1 might compete to, respectively, unwind and stabilize stable telomeric structures. Recently, competitive functions of Sgs1 and Pif1 during DNA replication have been suggested (WAGNER *et al.* 2006). However, since HR proteins are required to restore temperature resistance in *yku70Δ* when Pif1 is absent (Figure 3 and supplemental Figure

1), we speculate that Pif1 removes HR proteins from telomeres in cells lacking the Ku protein.

In this study, we uncovered a mechanism that prevents the activation of the cell cycle checkpoint at high temperatures in the *yku70Δ* strain, resulting in cell survival. The traditional means for achieving temperature resistance in this strain required either telomerase or a mutation resulting in telomere lengthening. We found that, in the absence nuclear Pif1, the telomeres of *yku70Δ* were lengthened, temperature resistance was restored, and cell cycle checkpoints were no longer activated. Our data, however, show that a HR-dependent pathway is ultimately more important to temperature resistance than telomere length.

We thank S. Lee (University of Texas at San Antonio) and M. Lichten of the National Institutes of Health (NIH) for helpful discussions; J. Haber (Brandeis University), W. Heyer (University of California at Davis), S. Jackson (University of Cambridge), N. Sugawara (Brandeis University), P. Sung (Yale University), and S. Teo (University of Cambridge) for providing plasmids, strains, and antibodies; and B. Berman at the National Human Genome Research Institute (NHGRI), S. Dunaway (Drew University), E. Hendrickson (University of Minnesota), H. Liaw (NHGRI), and A. Zhang (NHGRI) for comments on the manuscript. K. Myung thanks E. Cho for great support. This work was supported by the intramural research program of the NHGRI at the NIH (to K.M.).

LITERATURE CITED

- AYLON, Y., and M. KUPIEC, 2004 DSB repair: the yeast paradigm. *DNA Repair* **3**: 797–815.
- BAILEY, S. M., J. MEYNE, D. J. CHEN, A. KURIMASA, G. C. LI *et al.*, 1999 DNA double-strand break repair proteins are required to cap the ends of mammalian chromosomes. *Proc. Natl. Acad. Sci. USA* **96**: 14899–14904.
- BANERJEE, S., and K. MYUNG, 2004 Increased genome instability and telomere length in the *elg1*-deficient *Saccharomyces cerevisiae* mutant are regulated by S-phase checkpoints. *Eukaryot. Cell* **3**: 1557–1566.
- BANERJEE, S., S. SMITH and K. MYUNG, 2006 Suppression of gross chromosomal rearrangements by yKu70-yKu80 heterodimer through DNA damage checkpoints. *Proc. Natl. Acad. Sci. USA* **103**: 1816–1821.
- BARNES, G., and D. RIO, 1997 DNA double-strand-break sensitivity, DNA replication, and cell cycle arrest phenotypes of Ku-deficient *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **94**: 867–872.
- BASRAI, M. A., V. E. VELCULESCU, K. W. KINZLER and P. HIETER, 1999 NORF5/HUG1 is a component of the MEC1-mediated checkpoint response to DNA damage and replication arrest in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **19**: 7041–7049.
- BERTUCH, A. A., and V. LUNDBLAD, 2004 EXO1 contributes to telomere maintenance in both telomerase-proficient and telomerase-deficient *Saccharomyces cerevisiae*. *Genetics* **166**: 1651–1659.
- BOULE, J. B., L. R. VEGA and V. A. ZAKIAN, 2005 The yeast Pif1p helicase removes telomerase from telomeric DNA. *Nature* **438**: 57–61.
- BOULTON, S. J., and S. P. JACKSON, 1996 *Saccharomyces cerevisiae* Ku70 potentiates illegitimate DNA double-strand break repair and serves as a barrier to error-prone DNA repair pathways. *EMBO J.* **15**: 5093–5103.
- BOULTON, S. J., and S. P. JACKSON, 1998 Components of the Ku-dependent non-homologous end-joining pathway are involved in telomeric length maintenance and telomeric silencing. *EMBO J.* **17**: 1819–1828.
- BUDD, M. E., C. C. REIS, S. SMITH, K. MYUNG and J. L. CAMPBELL, 2006 Evidence suggesting that Pif1 helicase functions in DNA replication with the Dna2 helicase/nuclease and DNA polymerase delta. *Mol. Cell. Biol.* **26**: 2490–2500.
- CHEN, C., and R. D. KOLODNER, 1999 Gross chromosomal rearrangements in *Saccharomyces cerevisiae* replication and recombination defective mutants. *Nat. Genet.* **23**: 81–85.
- DE LANGE, T., 2002 Protection of mammalian telomeres. *Oncogene* **21**: 532–540.
- DOWNEY, M., R. HOULSWORTH, L. MARINGELE, A. ROLLIE, M. BREHME *et al.*, 2006 A genome-wide screen identifies the evolutionarily conserved KEOPS complex as a telomere regulator. *Cell* **124**: 1155–1168.
- DOWNS, J. A., and S. P. JACKSON, 2004 A means to a DNA end: the many roles of Ku. *Nat. Rev. Mol. Cell Biol.* **5**: 367–378.
- EVANS, S. K., M. L. SISTRUNK, C. I. NUGENT and V. LUNDBLAD, 1998 Telomerase, Ku, and telomeric silencing in *Saccharomyces cerevisiae*. *Chromosoma* **107**: 352–358.
- FALZON, M., J. W. FEWELL and E. L. KUFF, 1993 EBP-80, a transcription factor closely resembling the human autoantigen Ku, recognizes single- to double-strand transitions in DNA. *J. Biol. Chem.* **268**: 10546–10552.
- FELDMANN, H., and E. L. WINNACKER, 1993 A putative homologue of the human autoantigen Ku from *Saccharomyces cerevisiae*. *J. Biol. Chem.* **268**: 12895–12900.
- FISHER, T. S., A. K. TAGGART and V. A. ZAKIAN, 2004 Cell cycle-dependent regulation of yeast telomerase by Ku. *Nat. Struct. Mol. Biol.* **11**: 1198–1205.
- FRIESLAND, S., L. KANTER-LEWENSOHN, R. TELL, E. MUNCK-WIKLAND, R. LEWENSOHN *et al.*, 2003 Expression of Ku86 confers favorable outcome of tonsillar carcinoma treated with radiotherapy. *Head Neck* **25**: 313–321.
- GANGLOFF, S., J. P. McDONALD, C. BENDIXEN, L. ARTHUR and R. ROTHSTEIN, 1994 The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.* **14**: 8391–8398.
- GRAVEL, S., M. LARRIVEE, P. LABRECQUE and R. J. WELLINGER, 1998 Yeast Ku as a regulator of chromosomal DNA end structure. *Science* **280**: 741–744.
- GU, Y., S. JIN, Y. GAO, D. T. WEAVER and F. W. ALT, 1997 Ku70-deficient embryonic stem cells have increased ionizing radio-sensitivity, defective DNA end-binding activity, and inability to support V(D)J recombination. *Proc. Natl. Acad. Sci. USA* **94**: 8076–8081.
- HAN, H., R. J. BENNETT and L. H. HURLEY, 2000 Inhibition of unwinding of G-quadruplex structures by Sgs1 helicase in the presence of N,N'-bis[2-(1-piperidino)ethyl]-3,4,9,10-perylene-tetracarboxylic diimide, a G-quadruplex-interactive ligand. *Biochemistry* **39**: 9311–9316.
- HICKSON, I. D., 2003 RecQ helicases: caretakers of the genome. *Nat. Rev. Cancer* **3**: 169–178.
- HSU, H. L., D. GILLEY, E. H. BLACKBURN and D. J. CHEN, 1999 Ku is associated with the telomere in mammals. *Proc. Natl. Acad. Sci. USA* **96**: 12454–12458.
- HUBER, M. D., D. C. LEE and N. MAIZELS, 2002 G4 DNA unwinding by BLM and Sgs1p: substrate specificity and substrate-specific inhibition. *Nucleic Acids Res.* **30**: 3954–3961.
- IVESSA, A. S., J. Q. ZHOU and V. A. ZAKIAN, 2000 The *Saccharomyces* Pif1p DNA helicase and the highly related Rrm3p have opposite effects on replication fork progression in ribosomal DNA. *Cell* **100**: 479–489.
- JACO, I., P. MUNOZ and M. A. BLASCO, 2004 Role of human Ku86 in telomere length maintenance and telomere capping. *Cancer Res.* **64**: 7271–7278.
- JOYCE, M. V., and L. B. MCGOWN, 2004 Detection of G-quartet structure in a DNA aptamer stationary phase using a fluorescent dye. *Appl. Spectrosc.* **58**: 831–835.
- KROGH, B. O., and L. S. SYMINGTON, 2004 Recombination proteins in yeast. *Annu. Rev. Genet.* **38**: 233–271.
- LAHAYE, A., H. STAHL, D. THINES-SEMPOUX and F. FOURY, 1991 PIF1: a DNA helicase in yeast mitochondria. *EMBO J.* **10**: 997–1007.
- LAROCHE, T., S. G. MARTIN, M. GOTTA, H. C. GORHAM, F. E. PRYDE *et al.*, 1998 Mutation of yeast Ku genes disrupts the subnuclear organization of telomeres. *Curr. Biol.* **8**: 653–656.
- LEWIS, L. K., G. KARTHIKEYAN, J. W. WESTMORELAND and M. A. RESNICK, 2002 Differential suppression of DNA repair deficiencies

- of yeast rad50, mre11 and xrs2 mutants by EXO1 and TLC1 (the RNA component of telomerase). *Genetics* **160**: 49–62.
- LI, J. L., R. J. HARRISON, A. P. RESZKA, R. M. BROSH, JR., V. A. BOHR *et al.*, 2001 Inhibition of the Bloom's and Werner's syndrome helicases by G-quadruplex interacting ligands. *Biochemistry* **40**: 15194–15202.
- LINGNER, J., and T. R. CECHE, 1998 Telomerase and chromosome end maintenance. *Curr. Opin. Genet. Dev.* **8**: 226–232.
- LOVETT, S. T., and R. K. MORTIMER, 1987 Characterization of null mutants of the RAD55 gene of *Saccharomyces cerevisiae*: effects of temperature, osmotic strength and mating type. *Genetics* **116**: 547–553.
- MARINIGLE, L., and D. LYDALL, 2002 EXO1-dependent single-stranded DNA at telomeres activates subsets of DNA damage and spindle checkpoint pathways in budding yeast yku70Delta mutants. *Genes Dev.* **16**: 1919–1933.
- MIMORI, T., and J. A. HARDIN, 1986 Mechanism of interaction between Ku protein and DNA. *J. Biol. Chem.* **261**: 10375–10379.
- MYUNG, K., C. CHEN and R. D. KOLODNER, 2001a Multiple pathways cooperate in the suppression of genome instability in *Saccharomyces cerevisiae*. *Nature* **411**: 1073–1076.
- MYUNG, K., A. DATTA, C. CHEN and R. D. KOLODNER, 2001b SGS1, the *Saccharomyces cerevisiae* homologue of BLM and WRN, suppresses genome instability and homeologous recombination. *Nat. Genet.* **27**: 113–116.
- MYUNG, K., A. DATTA and R. D. KOLODNER, 2001c Suppression of spontaneous chromosomal rearrangements by S phase checkpoint functions in *Saccharomyces cerevisiae*. *Cell* **104**: 397–408.
- MYUNG, K., G. GHOSH, F. J. FATTAH, G. LI, H. KIM *et al.*, 2004 Regulation of telomere length and suppression of genomic instability in human somatic cells by Ku86. *Mol. Cell. Biol.* **24**: 5050–5059.
- NUGENT, C. I., G. BOSCO, L. O. ROSS, S. K. EVANS, A. P. SALINGER *et al.*, 1998 Telomere maintenance is dependent on activities required for end repair of double-strand breaks. *Curr. Biol.* **8**: 657–660.
- NUSSENZWEIG, A., C. CHEN, V. DA COSTA SOARES, M. SANCHEZ, K. SOKOL *et al.*, 1996 Requirement for Ku80 in growth and immunoglobulin V(D)J recombination. *Nature* **382**: 551–555.
- ONODA, F., M. SEKI, A. MIYAJIMA and T. ENOMOTO, 2000 Elevation of sister chromatid exchange in *Saccharomyces cerevisiae* sgs1 disruptants and the relevance of the disruptants as a system to evaluate mutations in Bloom's syndrome gene. *Mutat. Res.* **459**: 203–209.
- ONODA, F., M. SEKI, A. MIYAJIMA and T. ENOMOTO, 2001 Involvement of SGS1 in DNA damage-induced heteroallelic recombination that requires RAD52 in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **264**: 702–708.
- OUYANG, H., A. NUSSENZWEIG, A. KURIMASA, V. C. SOARES, X. LI *et al.*, 1997 Ku70 is required for DNA repair but not for T cell antigen receptor gene recombination in vivo. *J. Exp. Med.* **186**: 921–929.
- POLOTNIANKA, R. M., J. LI and A. J. LUSTIG, 1998 The yeast Ku heterodimer is essential for protection of the telomere against nucleolytic and recombinational activities. *Curr. Biol.* **8**: 831–834.
- PORTER, S. E., P. W. GREENWELL, K. B. RITCHIE and T. D. PETES, 1996 The DNA-binding protein Hdf1p (a putative Ku homologue) is required for maintaining normal telomere length in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **24**: 582–585.
- REEVES, W. H., 1987 Antinuclear antibodies as probes to explore the structural organization of the genome. *J. Rheumatol.* **14**(Suppl. 13): 97–105.
- RIHA, K., J. M. WATSON, J. PARKEY and D. E. SHIPPEN, 2002 Telomere length deregulation and enhanced sensitivity to genotoxic stress in Arabidopsis mutants deficient in Ku70. *EMBO J.* **21**: 2819–2826.
- SAMPER, E., F. A. GOYTISOLO, P. SLIJEPCJEVIC, P. P. VAN BUUL and M. A. BLASCO, 2000 Mammalian Ku86 protein prevents telomeric fusions independently of the length of TTAGGG repeats and the G-strand overhang. *EMBO Rep.* **1**: 244–252.
- SCHULZ, V., and V. A. ZAKIAN, 1994 The *Saccharomyces PIF1* DNA helicase inhibits telomere elongation and *de novo* telomere formation. *Cell* **76**: 145–155.
- SMITH, S., J. Y. HWANG, S. BANERJEE, A. MAJEED, A. GUPTA *et al.*, 2004 Mutator genes for suppression of gross chromosomal rearrangements identified by a genome-wide screening in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. USA* **101**: 9039–9044.
- SPAGNOLO, L., A. RIVERA-CALZADA, L. H. PEARL and O. LLORCA, 2006 Three-dimensional structure of the human DNA-PKcs/Ku70/Ku80 complex assembled on DNA and its implications for DNA DSB repair. *Mol. Cell* **22**: 511–519.
- SUGAWARA, N., X. WANG and J. E. HABER, 2003 In vivo roles of Rad52, Rad54, and Rad55 proteins in Rad51-mediated recombination. *Mol. Cell* **12**: 209–219.
- SUN, H., R. J. BENNETT and N. MAIZELS, 1999 The *Saccharomyces cerevisiae* Sgs1 helicase efficiently unwinds G-G paired DNAs. *Nucleic Acids Res.* **27**: 1978–1984.
- TEO, S. H., and S. P. JACKSON, 2001 Telomerase subunit overexpression suppresses telomere-specific checkpoint activation in the yeast yku80 mutant. *EMBO Rep.* **2**: 197–202.
- TSUKAMOTO, Y., J. KATO and H. IKEDA, 1997 Silencing factors participate in DNA repair and recombination in *Saccharomyces cerevisiae*. *Nature* **388**: 900–903.
- VEGA, L. R., M. K. MATEYAK and V. A. ZAKIAN, 2003 Getting to the end: telomerase access in yeast and humans. *Nat. Rev. Mol. Cell Biol.* **4**: 948–959.
- VEGA, L. R., J. A. PHILLIPS, B. R. THORNTON, J. A. BENANTI, M. T. ONIGBANJO *et al.*, 2007 Sensitivity of yeast strains with long G-tails to levels of telomere-bound telomerase. *PLoS Genet.* **3**: e105.
- WAGNER, M., G. PRICE and R. ROTHSTEIN, 2006 The absence of Top3 reveals an interaction between the Sgs1 and Pif1 DNA helicases in *Saccharomyces cerevisiae*. *Genetics* **174**: 555–573.
- WATT, P. M., E. J. LOUIS, R. H. BORTS and I. D. HICKSON, 1995 Sgs1: a eukaryotic homolog of E. coli RecQ that interacts with topoisomerase II in vivo and is required for faithful chromosome segregation. *Cell* **81**: 253–260.
- WEINERT, T. A., and L. H. HARTWELL, 1993 Cell cycle arrest of cdc mutants and specificity of the RAD9 checkpoint. *Genetics* **134**: 63–80.
- WELLINGER, R. J., A. J. WOLF and V. A. ZAKIAN, 1993 Origin activation and formation of single-strand TG1-3 tails occur sequentially in late S phase on a yeast linear plasmid. *Mol. Cell. Biol.* **13**: 4057–4065.
- WILLIAMSON, J. R., M. K. RAGHURAMAN and T. R. CECHE, 1989 Monovalent cation-induced structure of telomeric DNA: the G-quartet model. *Cell* **59**: 871–880.
- WU, X., and N. MAIZELS, 2001 Substrate-specific inhibition of RecQ helicase. *Nucleic Acids Res.* **29**: 1765–1771.
- ZAHLER, A. M., J. R. WILLIAMSON, T. R. CECHE and D. M. PRESCOTT, 1991 Inhibition of telomerase by G-quartet DNA structures. *Nature* **350**: 718–720.
- ZHOU, J., E. K. MONSON, S. TENG, V. P. SCHULZ and V. A. ZAKIAN, 2000 Pif1p helicase, a catalytic inhibitor of telomerase in yeast. *Science* **289**: 771–774.

Communicating editor: A. NICOLAS