Supplemental tables

Table S1. Yeast strains used in this study, related to Figures 2-4.

Strain	**Genotype**
MBY49	*MATα ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 hxt13::URA3*
MBY79	MBY49 *rrm3::His3MX6*
MBY132	MBY49 *sgs1::his5*$^{+}$
MBY136	MBY49 *rrm3::His3MX6 pif1-m2*
MBY161	MBY49 *hrq1::His3MX6*
KP313	MBY49 *pif1-m2*
MBY164	MBY49 *pif1-m2 hrq1::His3MX6*
KP351	MBY49 *pif1-m2 sgs1::His3MX6*
MBY167	MBY49 *hrq1::TRP1 rrm3::His3MX6*
MBY168	MBY49 *sgs1::his5*$^{+}$ *hrq1::TRP1*
MBY345	MBY49 *hrq1::HRQ1-His*$_6$*-NatMX*
MBY346	MBY49 *hrq1::hrq1-KA-His*$_6$*-NatMX*
MBY347	MBY49 *sgs1::his5*$^{+}$ *hrq1::hrq1-KA-His*$_6$*-NatMX*
MBY382	MBY49 *pif1-m2 hrq1::hrq1-KA-His*$_6$*-NatMX*
MBY385	MBY49 *hrq1::TRP1 pif1-m2 rrm3::His3MX6*
MBY386	MBY49 *hrq1::hrq1-KA-His*$_6$*-NatMX sgs1::sgs1-KA-TRP1*
MBY387	MBY49 *sgs1::sgs1-KA-TRP1*
MBY446	MBY49 *hrq1::His3MX6 sgs1::sgs1-KA-TRP1*
MBY464	*MATα/a ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 HXT13/hxt13::URA3 HRQ1/ hrq1::TRP1 PIF1/pif1-m2 RAD52/rad52::LEU2 SGS1/sgs1::His3MX6*
MBY465	*MATα/a ura3-52 lys2-801_amber ade2-101_ochre trp1Δ63 his3Δ200 leu2Δ1 HXT13/hxt13::URA3 HRQ1/ hrq1::hrq1- KA-His*$_6$*-NatMX PIF1/pif1-m2 RAD52/rad52::LEU2 SGS1/ sgs1::sgs1-KA-TRP1*
MBY505	MBY49 *pso2::TRP1*
MBY506	MBY49 *hrq1::His3MX6 pso2::TRP1*
KP386	*MATα/a Δho Δhml::ADE1 Δhmr:::ADE1 ade1 leu2-3 112 lus 5 ura 3-52 TLC/tlc1::KanMX SGS1/sgs1::His3MX6 HRQ1/hrq1::NatMX*
XS95-6C	*MATα rad52-1 his3-delta1 leu2-3 leu2-112 ura3-52 trp1-289(amber mutation) gal [cir0] (2-micron DNA minus [cir0] host strains (BC1))*

Table S2. Oligonucleotides used in this study, related to Figures 1-4.

Name	Sequence (5'-3')	Purpose
MB452	*5Biosg/GGGACGCGTCGGCCTGGCACGTCG GCCGCTGCGGCCAGGCACCCGATGGC/3Bio	Directionality substrate
MB453	CCGACGTGCCAGGCCGACGCGTCCCTTTTTTT TTTTTTTTTTTTTTTTTTTTTTT	Directionality substrate
MB454	TTTTTTTTTTTTTTTTTTTTGCCATCGGGTGC CTGGCCGCAGCGG	Directionality substrate
MB464	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	ssDNA binding
MB530	CGTACGCTCGAGTATCTCTTTTTTAATGATTT CATTTGTATGAGTATCGTCTTTCGTAGC	*HRQ1*
MB571	CATTATTACAACATCGACCTCTTCAGGTGCGT CCCTTATTTACCAACTAGCAGCTATCG	*hrq1*-KA mutagenesis
MB572	CGATAGCTGCTAGTTGGTAAATAAGGGACGCA CCTGAAGAGGTCGATGTTGTAATAATG	*hrq1*-KA mutagenesis
MB634	GACACGAAATTACAAAATGGAATATGTTCATA GGGTAGACG	Multiplex PCR band 5
MB653	CATGCATGTCATGAGCTGGTCTCATCCACAAT TCGAAAAGGGTGCCAACTGGTCTCATCCACAA TTCGAAAAGGGTGCCAACTGGTCTCATCCACA ATTCGAAAAGGGTGCCAACTGGTCTCATCCAC AATTCGAAAAGGGTGCCAACGGATCCATGGAG GAAGGACCTATCAAAAAGAAACTGAAGTC	*HRQ1* cloning
MB733	ACCGTTGTGCAACTGAGTGGACAACGTGTCAC TCACATAGCGTTC	Helicase assay fork substrate
MB734	GAACGCTATGTGAGTGACACCAACAGGTGAGT CAACGTGTTGCCA	Helicase assay fork substrate
MB915	GTGTGGGTGTGGTGTGGGTGTGGTGTGGGT	ssDNA binding
Int CIN8 R	GAAAATCGACATAATAAGAGTAGATTTCC	Multiplex PCR band 4
Int CIN8 F	GATTTGCGATAGCGTCGCTGCC	Multiplex PCR band 4
IG CAN1 F	GAGTTTGCTAGATTCATAAAAGCC	Multiplex PCR band 1
IG CAN1 R	CCGATAATGTCTGAGTTAGGTGAG	Multiplex PCR band 1
IHS	GAAAAGGATACAAAGGATATGAG	Multiplex PCR band 3
OHS	GTTTTCTTCGATTTGAAGGTGTTGG	Multiplex PCR bands 2 & 3
IG SOM1	CGAGGTCACGGACACATATACC	Multiplex PCR band 5
Int NPR2 Rev	CTTAGTTTAGAAATTTTGGCAATG	Multiplex PCR band 2
PCM1 For	CGATGAAGGTTGATTACGAGC	Multiplex PCR band 6
PCM1 Int	GAAGGCTCCTATAAAGAACG	Multiplex PCR band 6

*5Biosg/ = 5' biotinylation; /3Bio = 3' biotinylation.

Supplemental Experimental Procedures

Protein purification

Full-length *HRQ1* was PCR-amplified from *S. cerevisiae* genomic DNA using oligonucleotides MB653 and MB530 (Table S2), digested with *Bsp*HI and *Xho*I, and ligated into the *Nco*I and *Xho*I sites of pET21d to create pMB309. This cloning added an N-terminal 4x StrepII tag and a C-terminal 6x His tag to the translated product. The expression plasmid encoding the helicase dead *hrq1*-KA allele was created by site-direct mutagenesis of pMB309 using oligonucleotides MB571 and MB572.

Expression plasmids were transformed into Rosetta^{TM} 2(DE3) pLysS cells and selected for at 37°C on LB medium supplemented with 100 µg/mL ampicillin and 34 µg/mL chloramphenicol. Fresh transformants were used to inoculate one or more 5-mL LB cultures supplemented with antibiotics and incubated at 30°C for ~6 h with agitation. These starter cultures were then diluted 1:100 in ZYP-5052 autoinduction medium containing 1x trace metals mix (Studier, 2005), 100 µg/mL ampicillin, and 34 µg/mL chloramphenicol and incubated at 22°C with agitation to OD_{600} >3 (~18 h). Cells were harvested by centrifugation for 10 min in a GS-3 rotor at 5,000 rpm and 4°C. Cell pellets were weighed and frozen at -80°C prior to lysis or for long-term storage.

Frozen cells pellets were thawed at room temperature by stirring in 2 mL/g cell pellet buffer A (50 mM Na-HEPES (pH 8), 10% (v/v) glycerol, 300 mM NaCl, and 5 mM $MgCl_2$) supplemented with 1x protease inhibitor cocktail (Sigma), 20 µg/mL DNase I, and 2.5 µg/mL RNase A. Cells were lysed for 10-15 min at room temperature by adding *n*-dodecyl β-D-maltoside (DM; Sigma) to a final concentration of 0.05% (w/v) and 1x FastBreak (Promega) with gentle stirring. Subsequent steps were performed at 4°C.

The soluble fraction was clarified by centrifugation for 30 min in an SA-600 rotor at 13,000 rpm followed by filtering the supernatant through a 0.22-μm membrane. This mixture was then loaded onto a Strep-Tactin Sepharose gravity column (IBA) pre-equilibrated in buffer A. The column was washed with four column volumes each of buffer W1 (50 mM Na-HEPES, pH 8, 10% (v/v) glycerol, 600 mM NaCl, 5 mM $MgCl_2$, and 0.05% (v/v) DM) and W2 (50 mM Na-HEPES, pH 8, 10% (v/v) glycerol, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% (v/v) DM, and 5 mM ATP). Protein was eluted with three column volumes of buffer W3 (50 mM Na-HEPES, pH 8, 10% (v/v) glycerol, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% (v/v) DM, and 1 mM desthiobiotin). Column fractions were examined on 8% SDS-PAGE gels run at 20 V/cm and stained with Coomassie Brilliant Blue R-250 (BioRad).

Peak fractions were pooled and loaded onto a His60 Ni Gravity column (Clontech) pre-equilibrated in buffer W3. The column was washed with ten column volumes of buffer W4 (50 mM Na-HEPES, pH 8, 10% (v/v) glycerol, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% (v/v) DM, and 10 mM imidazole), and protein was eluted with six column volumes of W5 (50 mM Na-HEPES, pH 8, 10% (v/v) glycerol, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% (v/v) DM, and 400 mM imidazole). Fractions were analyzed by SDS-PAGE as above, and peak fractions were pooled and concentrated by centrifugation for 24 min at 14,000 g and 4°C in Amicon Ultra YM-30 units. The protein was then buffer-exchanged into storage buffer (25 mM Na-HEPES, pH 8.0, 30% glycerol, 50 mM NaOAc, pH 7.6, 25 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, and 0.01% DM) using Zebra Desalt spin columns (40K MWCO; Pierce), following the manufacturer's instructions. Typically, we obtained > 200 μg of purified Hrq1 from 25 g of cell pellet.
The hRecQ4 expression plasmid pGEX-RecQ4-His9 was a gift from Patrick Sung (Yale University). Full-length hRecQ4 was purified essentially as described (Macris et al., 2006). The

protein concentration and purity of the final preparations were determined on SYPRO orange (Sigma)-stained SDS-PAGE gels using known amounts of a standard protein for comparison. In all cases, protein purity was ≥ 95%. For some protein preparations, the N-terminal 4x *Strep*-II tag was removed by PreScission Protease (GE Healthcare) digestion (2 U protease/mL protein at 4°C overnight) prior to His60 column chromatography. In all cases, removal of the tag had little effect on subsequent protein purity and no effect on the *in vitro* activities examined.

Native gradient gels

Native gradient gels were poured using three solutions: Bottom (15% 37.5:1 acrylamide:bis-acrylamide, 10% glycerol, 20 mM Tris-HCl, pH 8.8, 200 mM glycine, and 0.1% (w/v) bromophenol blue); Top (3.5% 37.5:1 acrylamide:bis-acrylamide, 20 mM Tris-HCl, pH 8.8, and 200 mM glycine); and Stacker (3.5% 37.5:1 acrylamide:bis-acrylamide, 20 mM Tris-HCl, pH 6.8, and 200 mM glycine). The gradients were formed in disposable 5-mL pipettes by first pipetting 1.5 mL Top solution, then 1.5 mL Bottom solution, and gently mixing the two phases by bubbling air through the solutions. The gradient solutions were then carefully pipetted between vertical gel casting plates and allowed to polymerize for ≥30 min at room temperature under a layer of water-saturated butanol. The butanol was subsequently flushed away with water, and a layer of stacking gel was added using the Stacking solution, into which the lane comb was inserted. The stacking gel was allowed to polymerize for 30 min at room temperature or overnight at 4°C.

Multiplex PCR

Genomic DNA isolated was from *S. cerevisiae* strains before and after GCR events using a MasterPure Yeast DNA Isolation kit (Epicentre Biotechnologies) and analyzed by multiplex

PCR as described in (Paeschke et al., 2013). Briefly, multiplex PCR was performed using the indicated primer pairs in Table 2 and the following cycling parameters: initial denaturation for 5 min at 95°C; 35 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 10 min. PCR products (20 μL/reaction) were run at 90 V on 2.5% agarose gels containing ethidium bromide and visualized by UV transillumination.

Gel filtration

Gel filtration analysis of Hrq1 was performed essentially as described in (Bochman and Schwacha, 2007). Briefly, a 1-ml Sephacryl 300 HR (Sigma) column was equilibrated in Hrq1 storage buffer and calibrated with standard molecular weight markers, including blue dextran (2,000 kDa), thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), alcohol dehydrogenase (150 kDa), albumin (66 kDa), and carbonic anhydrase (29 kDa). Approximately 1 μg purified protein was then subjected to analytical gel filtration chromatography run by gravity flow at room temperature. One-drop fractions were collected and analyzed by SDS-PAGE and staining with Sypro Orange and quantified using a Typhoon 9410 scanner and Image Gauge software.

Western blotting

Cell extracts for western blotting were prepared as described in (Kushnirov, 2000). Briefly, cells were grown overnight in YEPD liquid medium at 30°C with aeration. Then, 1 mL of OD_{600} = 2.5 cells was harvested, resuspended in 200 μL 0.1 N NaOH, incubated at room temperature for 5 min, pelleted, resuspended in 50 μL SDS-PAGE sample buffer, boiled for 3 min, and pelleted again. Subsequently, 6 μL of the supernatants was loaded onto 8% (37.5:1 polyacrylamide:bis-acrylamide) SDS-PAGE gels and run at 20 V/cm. The proteins were

transferred to a nitrocellulose membrane at 4°C and blocked with 5% non-fat milk in TBST at room temperature using standard protocols. The blot was probed with 1:500 anti-His antibody (37-2900, Invitrogen) to detect His_6-tagged Hrq1 and Hrq1-KA, 1:1000 anti-Sgs1 antibody (yC-17, Santa Cruz), or 1:1000 anti-tubulin antibody (G094, ABM) and visualized with HRP-conjugated secondary antibodies and ECL detection reagents (GE Healthcare).

Single-stranded DNA (ssDNA) binding

The ssDNA binding substrates were 5' end labeled with T4 PNK and γ^{32}P-ATP, and the ssDNA was separated from free label using an illustra ProbeQuant G-50 micro column (GE Healthcare) following the manufacturer's instructions. Binding reactions (10 μL) were performed in 1x binding buffer and contained 0.1 nM radiolabeled substrate and protein as indicated . The reactions were incubated at 30°C for 30 min, and then 6x native loading buffer (15% (w/v) Ficoll (type 400), 0.25% bromophenol blue, and 0.25% xylene cyanol) was added to a 1x final concentration. The samples were then separated on 6% 19:1 acrylamide:bis-acrylamide gels in 1x TBE buffer at 100 V/cm for 45 min, dried under vacuum, and imaged and quantified using a Typhoon 9410 scanner and Image Gauge software.

Supplemental references

Bochman, M.L., and Schwacha, A. (2007). Differences in the single-stranded DNA binding activities of MCM2-7 and MCM467: MCM2 and MCM5 define a slow ATP-dependent step. J Biol Chem *282*, 33795-337804.

Boule, J., Vega, L., and Zakian, V. (2005). The Yeast Pif1p helicase removes telomerase from DNA. Nature *438*, 57-61.

Kushnirov, V.V. (2000). Rapid and reliable protein extraction from yeast. Yeast *16*, 857-860.

Paeschke, K., Capra, J.A., and Zakian, V.A. (2011). DNA Replication through G-Quadruplex Motifs Is Promoted by the Saccharomyces cerevisiae Pif1 DNA Helicase. Cell *145*, 678-691.