Mph1p promotes gross chromosomal rearrangement through partial inhibition of homologous recombination

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Gross chromosomal rearrangement (GCR) is a type of genomic instability associated with many cancers. In yeast, multiple pathways cooperate to suppress GCR. In a screen for genes that promote GCR, we identified *MPH1*, which encodes a 3′–5′ DNA helicase. Overexpression of Mph1p in yeast results in decreased efficiency of homologous recombination (HR) as well as delayed Rad51p recruitment to double-strand breaks (DSBs), which suggests that Mph1p promotes GCR by partially suppressing HR. A function for Mph1p in suppression of HR is further supported by the observation that deletion of both *mph1* and *srs2* synergistically sensitize cells to methyl methanesulfonate-induced DNA damage. The GCR-promoting activity of Mph1p appears to depend on its interaction with replication protein A (RPA). Consistent with this observation, excess Mph1p stabilizes RPA at DSBs. Furthermore, spontaneous RPA foci at DSBs are destabilized by the *mph1*Δ mutation. Therefore, Mph1p promotes GCR formation by partially suppressing HR, likely through its interaction with RPA.

Introduction

Gross chromosomal rearrangements (GCRs) comprise one class of genomic instabilities found in many cancers. GCRs include translocations, deletions of chromosome arms, interstitial deletions, inversions, amplifications, chromosome end-to-end fusions, and aneuploidy (Kolodner et al., 2002; Lengauer, 2005; Teixeira and Heim, 2005). Chromosomes from cells carrying mutations in cancer susceptibility genes showed a large number of GCRs, which suggests that GCRs could be a means to achieve the multiple mutations necessary for carcinogenesis. GCR suppression studies using yeast as a model organism have demonstrated that multiple pathways cooperate to suppress GCRs (Myung et al., 2001c; Kolodner et al., 2002; Motegi and Myung, 2007).

Homologous recombination (HR) is thought to be a vital pathway for suppressing GCR because it plays a crucial role in the repair of DNA breaks (Myung et al., 2001a; Kolodner et al., 2002; Symington, 2002). Interestingly, in addition to a GCR suppression role, detailed genetic studies of HR and GCRs have revealed that the restrained recruitment of HR proteins can promote GCR formation (Myung et al., 2001a; Motegi et al., 2006). Thus, there should be mechanisms that determine when HR proteins participate in appropriate DNA repair and when they are involved in the misrepair (i.e., GCR formation). DNA helicases may be involved in such mechanisms.

DNA helicases/translocases melt DNA duplexes and remove proteins from DNA during DNA replication, HR, and DNA repair (Krejci et al., 2003; Veaute et al., 2003; Opresko et al., 2004; Cheok et al., 2005). DNA helicase/translocase dysfunction is frequently associated with chromosome instability and carcinogenesis. For example, cancer-prone diseases such as Bloom, Werner, and Rothmund-Thompson syndromes are caused by mutations in BLM, WRN, and RTS helicases, respectively (Opresko et al., 2004; Cheok et al., 2005). Their yeast homologue,

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Abbreviations used in this paper: 5-FOA, 5-fluoroorotic acid; ChIP, chromatin immunoprecipitation; DSB, double-strand break; FA, Fanconi anemia; GCR, gross chromosomal rearrangement; HR, homologous recombination; MMS, methyl methanesulfonate; NHEJ, nonhomologous end joining; RPA, replication protein A; YPD, yeast extract peptone-dextrose.

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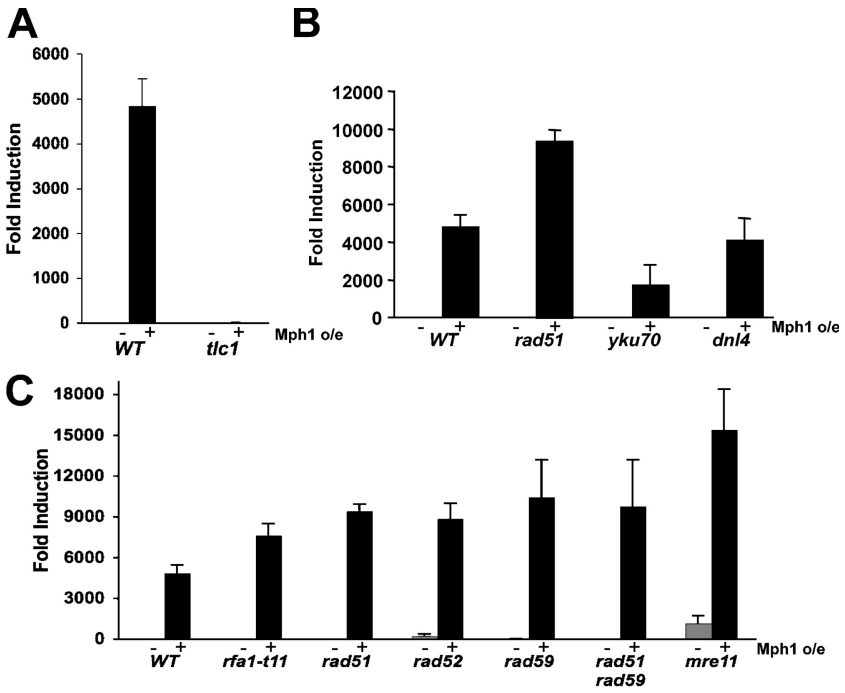

Figure 1. **A high level of Mph1p enhances GCR.** (A) GCR formation caused by excess Mph1p depends on telomerase activity. (B) Defects in HR but not NHEJ enhanced GCR rates synergistically when Mph1p was highly expressed. The *yku70Δ* mutation decreased the level of GCR enhancement. (C) Inactivation of HR with Mph1p overexpression synergistically enhanced GCRs. o/e, overexpression; WT, wild type. − (gray) and + (black) indicate without and with Mph1p overexpression, respectively. The GCR rates are provided in Table S1 (available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). Rates are presented as the mean of two median values with standard deviation.

Sgs1p, suppresses GCRs (Myung et al., 2001b; Schmidt et al., 2006). The yeast Pif1 helicase assists in telomere maintenance and DNA replication (Schulz and Zakian, 1994; Ivessa et al., 2000; Zhou et al., 2000; Budd et al., 2006) and suppresses GCR (Myung et al., 2001a; Schulz and Zakian, 1994). The yeast Srs2p helicase suppresses Rad51p-dependent HR (Krejci et al., 2003; Opresko et al., 2004) and promotes general GCR (Motegi et al., 2006). In addition, mutations in FANCM and BACH1 (also known as BRIP1) helicases are the cause of cancer prone phenotypes of Fanconi anemia (FA) group M and J patients, respectively (Kennedy and D'Andrea, 2005; Levran et al., 2005; Meetei et al., 2005; Mosedale et al., 2005).

FA is a genomic instability disorder, clinically characterized by congenital abnormalities, progressive bone marrow failure, and a predisposition to malignancy (Kennedy and D'Andrea, 2005). The FA core complex consists of 13 proteins participating in the DNA damage response network with BRCA1 and BRCA2. FANCM, a newly identified component of this complex, is structurally similar to the Archaeal bacterial protein Hef, which may process stalled replication forks (Komori et al., 2004).

Mph1p is a putative *Saccharomyces cerevisiae* homologue of FANCM and has been implicated in an HR-dependent pathway (Schurer et al., 2004; Onge et al., 2007). Mph1p has single-stranded DNA-dependent ATPase, DEAH, and 3′–5′ DNA helicase motifs (Prakash et al., 2005). Mutation in *MPH1* increases the forward mutation rate at the *CAN1* locus and enhances the reversion of *trp1-289* harboring an amber mutation (Scheller et al., 2000). The *mph1Δ* strain is sensitive to various DNA-damaging agents including methyl methanesulfonate (MMS), 4-nitroquinoline 1-oxide, and camptothecin (Scheller et al., 2000; Schurer et al., 2004). The *mph1Δ* mutation does not impair mitotic heteroallelic recombination. Nevertheless, it elevates spontaneous allelic recombination frequency in a strain carrying a mutation in another helicase gene, *SGS1* (Schurer et al., 2004). Recently, a genome-wide genetic interaction study suggested that Mph1p could function in HR (Onge et al., 2007). However, more work is clearly needed to better define Mph1's role in DNA repair.

Cancers are often accompanied by overexpression of multiple oncogenes. Despite many studies identifying pathways that suppress GCR (Kolodner et al., 2002; Motegi and Myung, 2007), little is known about activation mutations that enhance GCRs. To discover proteins that enhance GCR when overexpressed, we screened a yeast overexpression library and found Mph1p. Mph1p enhanced GCR rates ∼4,800-fold when overexpressed compared with the normal level of expression. Interestingly, the high levels of Mph1p enhanced GCR formation through the partial inhibition of the Rad52p-dependent HR. GCRs caused by excess Mph1p are dependent on the interaction of Mph1p with replication protein A (RPA). Consistently, excess Mph1p increased RPA accumulation at double strand breaks (DSBs). In contrast, the *mph1Δ* mutation caused reduction of spontaneous GCR and RPA foci formation. In addition, the *mph1Δ* mutation enhanced MMS sensitivity synergistically with the *srs2Δ* mutation, which suggests that like Srs2p, Mph1p may function at the level of suppressing damage-induced Rad52p-dependent HR. Collectively, these results suggest that Mph1p promotes GCR formation by partially suppressing HR through its interaction with RPA.

Results

Mph1 promotes GCR

The *S. cerevisiae* chromosome V GCR assay has been extensively used to identify genes that suppress GCRs (Kolodner et al., 2002; Motegi and Myung, 2007). In contrast, only a small number of genes have been identified as genes promoting GCR (Myung et al., 2001a; Lengronne and Schwob, 2002; Tanaka and Diffley, 2002; Hwang et al., 2005). To find genes that promote GCR formation, we transformed a *pif1Δ* strain (RDKY4399) with yeast

Table I. **The *mph1Δ* mutation caused different effects in GCR generated by different GCR mutator mutations**

Relevant genotype	WT		*mph1Δ*	
	Strain number	GCR rate CAN^r–5-FOA^r	Strain number	GCR rate CAN^r–5-FOA^r
Wild type	RDKY3615	3.5×10^{-10} (1)	YKJM1450	$<2.7 \times 10^{-10}$ (1)
rad5Δ	YKJM1386	9.0×10^{-8} (257)	YKJM3259	$<3.5 \times 10^{-10}$ (1)
rad18Δ	YKJM1389	7.1×10^{-8} (202)	YKJM3261	$<3.5 \times 10^{-10}$ (1)
mec1Δ	RDKY3735	4.6×10^{-8} (131)	YKJM2698	3.1×10^{-8} (89)
rfa1-t33	RDKY3617	4.7×10^{-7} (1,342)	YKJM2701	1.4×10^{-7} (400)
mre11Δ	RDKY3633	2.2×10^{-7} (629)	YKJM2875	1.4×10^{-7} (400)
rad27Δ	RDKY3630	4.4×10^{-7} (1,257)	YKJM2703	6.6×10^{-7} (1,886)
pif1-m2	RDKY4343	5.8×10^{-8} (166)	YKJM3355	8.8×10^{-8} (251)

All strains are isogenic with the wild-type strain RDKY3615 (*MATa, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8, hxt13::URA3*) with the exception of the indicated mutations. Numbers in parentheses indicate the rate relative to the wild type. The *mec1Δ* mutation has the *sml1Δ* mutation to suppress lethality. CAN^r–5-FOA^r, canavanine- and 5-FOA–resistant.

2μ genomic DNA libraries and monitored GCRs of individual transformants by replica patch testing. We used the *pif1Δ* strain to improve the sensitivity of the screening because the *pif1Δ* mutation synergistically increases GCR rates when it is combined with almost all known mutations enhancing GCRs (Myung et al., 2001a; Smith et al., 2004).

Approximately 1,200 individual colonies were patched as 1 × 1 cm squares, in duplicate. Because the mean insert size of this library is ∼10 kb, this number covers ∼64% of yeast genes according to the Clarke and Carbon formula, which calculates the probability of genome coverage (Clarke and Carbon, 1976). We selected 52 putative clones and retested each of them with six additional patches from the original plates. Plasmids from 21 clones still producing higher GCRs were recovered and amplified in *Escherichia coli* before being transformed back into yeast. 13 clones that reproducibly enhanced GCR after retransformation were selected, and both ends of the insert from each plasmid were sequenced.

The clone that yielded the highest GCR enhancement carried a plasmid with *SGN1*, *MPH1*, and two hypothetical open reading frames, *YIL001w* and *YIR003w*, as an insert. Sgn1p functions in RNA translation and is unlikely to be linked to GCR formation. Thus, we hypothesized that the GCR enhancement caused by this plasmid was caused by excess Mph1p. To test this hypothesis, we subcloned the full-length *MPH1* gene into the multi-copy 2μ plasmid p42K-TEF, which expressed *MPH1* from a strong TEF promoter. Mph1p overexpression increased GCR rates nearly 5,000-fold in the wild-type strain (RDKY3615) compared with the vector control (Fig. 1 and Table S1, available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). Rearrangement structures from 20 independent clones carrying independent GCRs were all broken chromosomes healed by de novo telomere addition requiring telomerase. Consistent with this result, the inactivation of the telomerase RNA subunit *TLC1* completely abolished Mph1p-induced GCRs (Fig. 1 A and Table S1).

To address whether Mph1p promotes GCR under physiological expression conditions, we chose several GCR mutator strains to understand whether the *mph1Δ* mutation could reduce GCR rates enhanced by these GCR mutator mutations. The *mph1Δ* mutation in both *rad5Δ* and *rad18Δ* strains reduced GCR rates to a level indistinguishable from the wild type and partially reduced GCR rates in *mec1Δ* and *rfa1-t33* strains (Table I). However, reductions of GCR rates by the *mph1Δ* mutation were not observed in other GCR mutator *mre11Δ*, *rad27Δ*, or *pif1-m2* strains (Table I). Therefore, Mph1p promotes some pathways of GCR formation under physiological expression conditions.

Mph1p promotes GCR through partial suppression of HR

GCRs have been linked to multiple pathways, including two major pathways to repair DNA DSBs: HR and nonhomologous end joining (NHEJ; Kolodner et al., 2002). To evaluate the effects of HR and NHEJ on GCRs induced by excess Mph1p, *MPH1* was overexpressed in strains defective in either HR (*rad51Δ*) or NHEJ (*dnl4Δ*), and GCR rates were monitored. In contrast to similar enhancement of GCRs in the *dnl4Δ* strain, excess Mph1p doubled GCRs in the *rad51Δ* strain compared with the wild type (Fig. 1 B and Table S1). Mutations in other HR genes including *RAD52*, *RAD59*, and *MRE11* or the *rfa1-t11* mutation similarly enhanced GCRs when Mph1p was overexpressed (Fig. 1 C and Table S1). Types of GCRs were determined in a total of 72 clones: 16 from *mre11Δ*, 21 from *rad51Δ*, 17 from *rad52Δ*, and 18 from *rad59Δ*. All clones had broken chromosomes healed by de novo telomere addition. Because inactivation of NHEJ did not reduce GCRs caused by excess Mph1p, and inactivation of HR even enhanced GCRs caused by excess Mph1p, neither NHEJ nor HR promote GCRs when Mph1p is overexpressed. Although loss of one NHEJ factor yKu70p reduced Mph1p-induced GCRs by half (Fig. 1 B and Table S1), this reduction likely reflects an inefficient recruitment of telomerase (Myung et al., 2001a; Banerjee et al., 2006) rather than loss of the NHEJ function by the *yku70Δ* mutation.

Interestingly, GCRs were further enhanced when Mph1p was overexpressed in HR-deficient strains (Fig. 1 C and Table S1). This result indicates that HR may suppress GCRs caused by excess Mph1p. We thus hypothesized that Mph1p-mediated GCRs arise after partial suppression of HR accompanied by simultaneous activation of a GCR pathway by Mph1. To test this hypothesis, we examined the effect of excess Mph1p on the

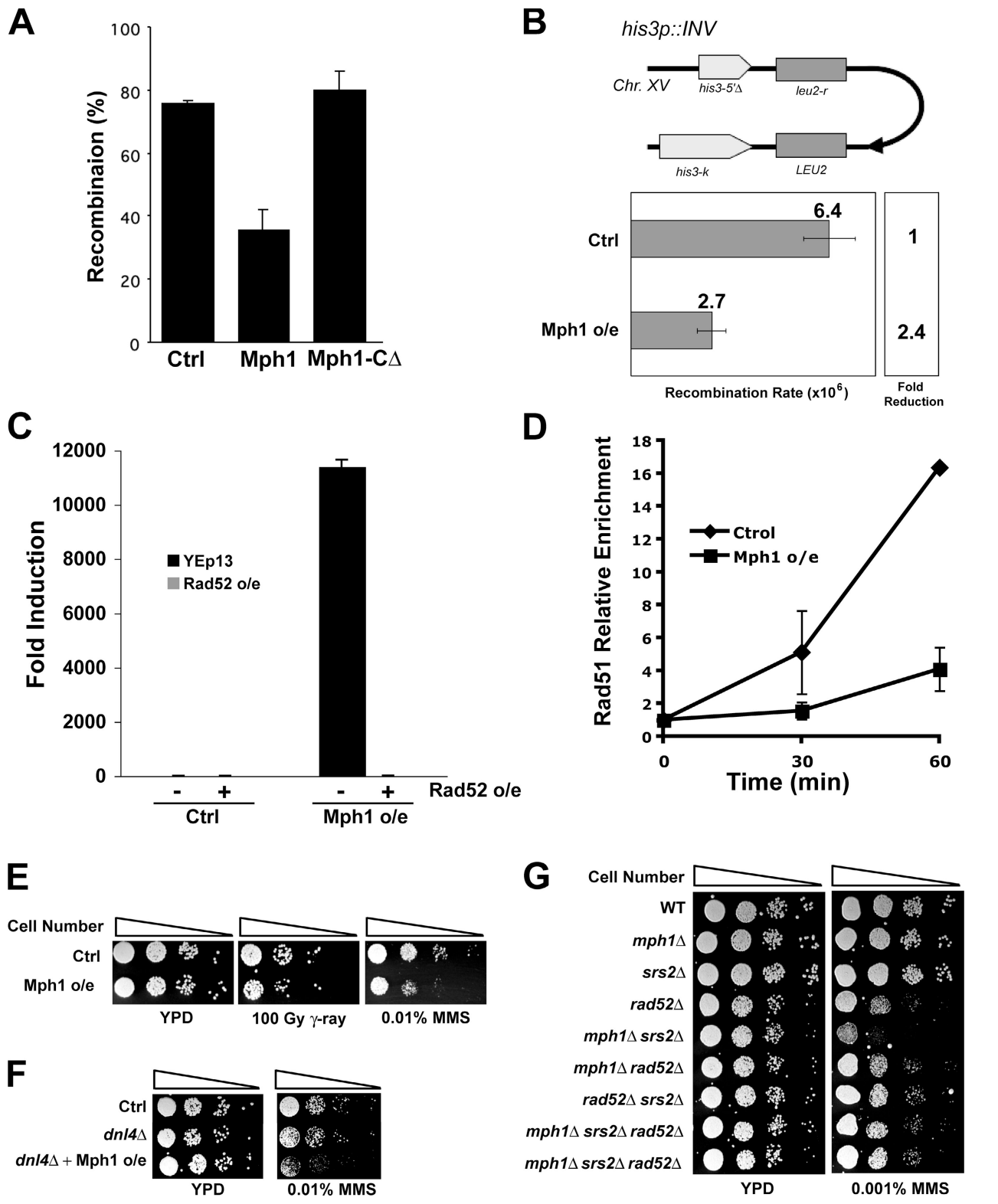

Figure 2. **Excess Mph1p down-regulates HR.** (A) High expression of Mph1p reduced mating type switching frequency using JKM161 with different plasmids (*Δho HMLalpha MATa Δhmr::ADE1 ade1-100 leu2-3,112 lys5 trp1::hisg ura3-52 ade3::GAL-HO endonuclease his-*). (B) Excess Mph1p reduces the spontaneous recombination rate. (top) A schematic diagram of *his3* inverted repeat spontaneous recombination assay using M137-11B with different plasmids (*MATa can1-100 his3p::INV leu2 lys2-128 trp1 ura3*). (bottom) A graphic presentation of spontaneous recombination rates of cells carrying control (Ctrl) or Mph1p overexpression (o/e) plasmids. (C) GCR enhancement by excess Mph1p was completely blocked by Rad52p cooverexpression. (D) Excess Mph1p slowed down Rad51p recruitment to DSB. ChIP was performed using JKM161 with different plasmids. (E) Excess Mph1p made cells sensitive to γ irradiation and MMS. (F) Excess Mph1p made the *dnl4Δ* strain sensitive to MMS. (G) Strains carrying both *mph1Δ* and *srs2Δ* mutations showed synergistic sensitivity to MMS compared with strains carrying each single mutation. Rates are presented as the mean of two median values with standard deviation.

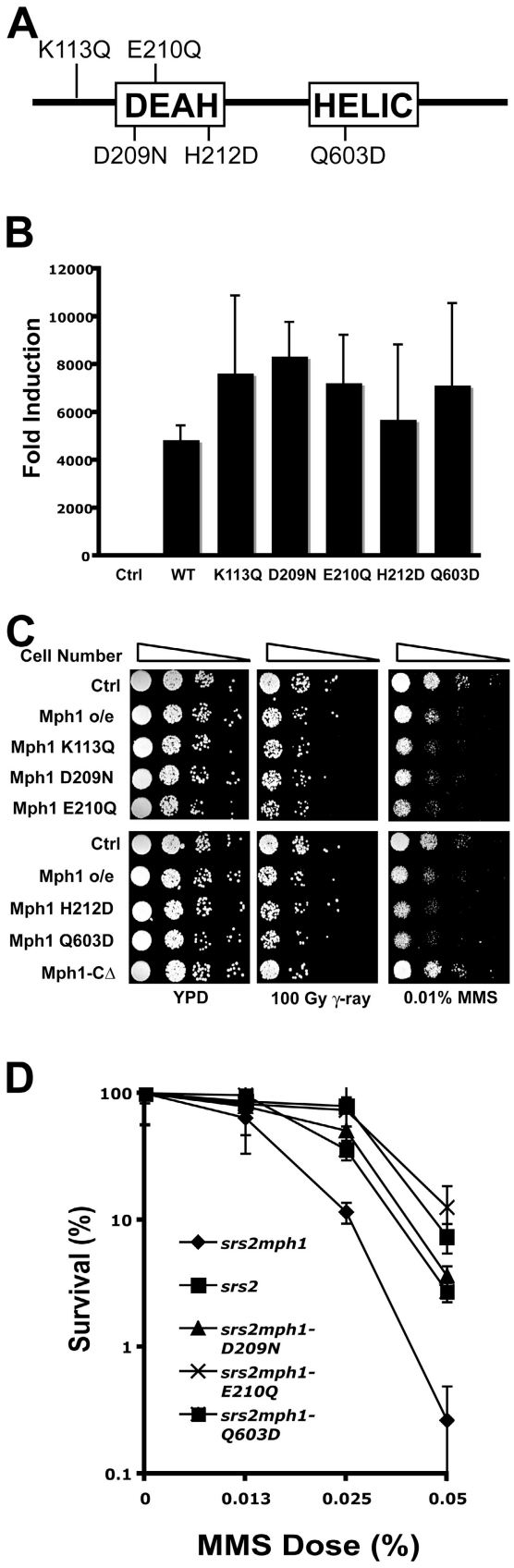

Figure 3. **ATPase, DEAH, or helicase motifs of Mph1p are dispensable for GCR-promoting activity and synergistic sensitivity to MMS with the *srs2Δ* mutation.** (A) Locations of mutations used in this study. (B) The overexpression of ATPase, DEAH, or helicase mutant Mph1p proteins still showed strong GCR enhancement similar to what was achieved by the overexpression of wild-type Mph1p. The GCR rates are provided in Table S2 (available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). (C) γ ray and MMS sensitivities caused by excess Mph1p remain when mutant Mph1p proteins were overexpressed. (D) The synergistic MMS sensitivity by the *mph1Δ* mutation in the *srs2Δ* strain was rescued by the Mph1ps carrying a mutation in the helicase or DEAH motifs. Rates are presented as the mean of two median values with standard deviation.

mating type switch recombination in a strain expressing *HO* endonuclease under a galactose-inducible promoter and the intact donor sequence. We found that excess Mph1p substantially reduced the yeast mating type switch recombination (Fig. 2 A). Furthermore, spontaneous HR between inverted repeats was reduced when Mph1p was overexpressed (Fig. 2 B). Detailed analysis of recombination events indicates that there were no significant differences in rates of single strand annealing or short-track gene conversion events; however, a significant decrease in large-track gene conversion with crossover events was observed (Table S2, available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). Recently, the in vitro branch migration activity of FANCM, a mammalian putative Mph1p homologue, was observed (Gari et al., 2008). Because the branch migration activity reduces crossover, both Mph1p and FANCM could have similar activity for genomic stability. Previous studies described that excess Rad52p can partially offset HR deficiency in certain mutants (Firmenich et al., 1995). We thus measured GCR rates when Rad52p and Mph1p were simultaneously overexpressed. Rad52p overexpression completely abolished the GCR enhancement caused by excess Mph1p (Fig. 2 C). The expression level of Mph1p was not affected by cooverexpression of Rad52p (unpublished data). Together, these results indicate that excess Mph1p partially compromises both DSB-induced and spontaneous HR. In further support of this idea, we observed slightly enhanced sensitivity of Mph1p-overexpressing cells to both γ-ray irradiation and MMS; we also observed the synergistic increase of sensitivity to MMS in the NHEJ-deficient *dnl4Δ* strain (Fig. 2, E and F).

We hypothesized that excess Mph1p could interfere with the early decision step for HR repair. To test this hypothesis, the kinetics of Rad51p recruitment to an induced single DSB by *HO* endonuclease was monitored using chromatin immunoprecipitation (ChIP) analysis with Rad51p antibody in the presence of excess Mph1p. Excess Mph1p delayed Rad51p recruitment to the DSB, which implies that Mph1p indeed inhibits HR before or at the step of Rad51p filament formation (Fig. 2 D).

The Srs2p helicase removes Rad51p from single-stranded DNA to suppress HR repair (Krejci et al., 2003; Veaute et al., 2003). Delayed recruitment of Rad51p to the DSB (Fig. 2 D) suggested that Mph1p could function at the similar step with Srs2p. To test this hypothesis, the sensitivity of *mph1Δ*, *srs2Δ*, and *mph1Δ srs2Δ* strains to MMS and hydroxyurea was tested. In support of a similar function of Srs2p and Mph1p, we observed the synergistic sensitivity of the *mph1Δ srs2Δ* double mutant strain to both DNA-damaging agents (Fig. 2 G and unpublished data). Interestingly, the synergistic MMS sensitivity was partially rescued by the *rad52Δ* mutation (Fig. 2 G), which

Table II. **Different *mph1* mutations affect differently in GCR generated by the *rad5Δ* mutation**

Strain number	Plasmid	Mph1	GCR rate CAN^r–5-FOA^r
YKJM4810	pRS313 (HIS3)	Deletion	$<3.4 \times 10^{-9}$ (9)
YKJM1386	None	Wild type	9.0×10^{-8} (257)
YKJM4808	pKJM582 (HIS3)	H212D	1.4×10^{-8} (40)
YKJM4806	pKJM588 (HIS3)	Q603D	1.2×10^{-8} (34)
YKJM4804	pKJM584 (HIS3)	E210Q	1.8×10^{-8} (51)
YKJM4802	pKJM586 (HIS3)	D209N	1.7×10^{-8} (49)
YKJM4800	pKJM590 (HIS3)	K113Q	2.8×10^{-8} (80)
YKJM4825	p41k-CYC (KAN)	Deletion	$<2.1 \times 10^{-9}$ (6)
YKJM4827	pKJM781 (KAN)	Wild type	6.3×10^{-8} (180)
YKJM4829	pKJM937 (KAN)	CΔ	$<1.6 \times 10^{-9}$ (5)

YKJM4800, 4802, 4804, 4806, 4808, and 4810 strains are isogenic (*MATa, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8, hxt13::URA3, mph1::KAN, rad5::TRP1*) with the exception of the plasmid expressing different Mph1 indicated in the plasmid and Mph1 columns. YKJM1386 is isogenic, with the exception of carrying wild-type Mph1 in the genome. YKJM4825, 4827, and 4829 strains are isogenic (*MATa, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2ΔBgl, hom3-10, ade2Δ1, ade8, hxt13::URA3, mph1::TRP1, rad5::HIS3*) except for the transformed plasmid expressing Mph1 indicated in the plasmid and Mph1 columns. Numbers in parentheses in the plasmid column indicate the marker in the plasmid used. Numbers in parentheses in the GCR rate column indicate the rate relative to the wild type. CAN^r–5-FOA^r, canavanine- and 5-FOA–resistant.

suggests that in the absence of Mph1p and Srs2p, Rad52p-dependent HR may cause cell death in the presence of MMS.

The ATPase, DEAH, and helicase activities of Mph1p is dispensable for promoting GCR formation

Mph1p has three noticeable motifs: an ATPase, a DEAH, and a helicase motif (Fig. 3 A). To determine the extent of each motif's involvement in GCR enhancement, five Mph1p mutant proteins, each having an inactivating point mutation in one of three motifs (*K113Q* mutation in the ATPase motif; *D209N*, *E210Q*, and *H212D* mutations in the DEAH motif; and the *Q603D* mutation in the helicase motif), were overexpressed, and the GCR rates were monitored. In contrast to their inabilities to complement the *CAN1* locus mutator phenotype of the *mph1* strain (Scheller et al., 2000), the overexpression of these mutant Mph1p proteins could enhance GCRs to a similar level as wild-type Mph1p (Fig. 3 B and Table S3, available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). Furthermore, overexpression of these mutant Mph1p proteins, like wild-type Mph1p, sensitized cells to γ-irradiation and MMS (Fig. 3 C). Therefore, elevated GCR rates and DNA damage sensitivity do not appear to result from hyperactivation of Mph1p's helicase or ATPase activities.

GCRs in the *rad5Δ* strain were dependent on Mph1p (Table I). To determine if GCRs promoted by Mph1p in the *rad5Δ* strain require its ATPase or helicase functions, we measured GCRs in the *rad5Δ* strain expressing various mutant Mph1p proteins from a single copy plasmid. The expression of mutant Mph1p proteins significantly induced GCRs in the *rad5Δ* strain (Table II), although to a lesser extent than wild-type Mph1p. Therefore, Mph1p-dependent GCRs in the *rad5Δ* strain partially require Mph1p's ATPase/helicase activity.

The *mph1Δ srs2Δ* strain showed higher sensitivity to MMS compared with strains having either single mutation (Fig. 2 F). We examined whether such synergistic sensitivity was caused by defects in the DEAH or the helicase motif. The introduction of mutant Mph1p restored MMS resistance similar to the Mph1p wild type (Fig. 3 D). Therefore, the loss of activities associated with these domains is not responsible for the hyper-MMS sensitivity of *mph1Δ srs2Δ*.

The C terminus of Mph1p interacts with RPA and is important for promoting GCR

To better understand the mechanism of Mph1p-mediated GCRs, yeast clones carrying a randomly mutagenized *MPH1* overexpression plasmid were screened for their ability to induce GCRs. One mutant clone showed almost no GCR enhancement when it was overexpressed in the wild type (Fig. 4 A). A single adenine deletion from the eight-adenine repeats between nucleotides 2,852 and 2,859 of the *MPH1* gene in this mutant clone created a frame-shift mutation causing amino acid changes from valine-lysine to leucine-STOP at positions 914 and 915. The mutant protein, Mph1-CΔ mutant, is 39 amino acids shorter than the wild type because of the premature termination codon (Fig. 4 B). The Mph1-CΔ protein showed a similar expression level to wild-type Mph1 when we compared the expression of N-terminally Flag-tagged variants (Fig. 4 C, bottom left). Despite high expression, Mph1-CΔ overexpression failed to increase GCRs. Unlike wild-type Mph1p, excess Mph1-CΔ did not interfere with mating type switch recombination (Fig. 2 A) or cause MMS sensitivity (Fig. 3 C), and single copy expression of Mph1-CΔ did not reverse the suppression of GCR in the *rad5Δ mph1Δ* strain (Table II). Collectively, these data illustrate that the C terminus of Mph1p is required to promote GCRs.

The strong suppression of GCR by Rad52p cooverexpression (Fig. 2 C) suggests that excess Mph1p might interact with a protein functioning at the early stages of HR. We hypothesized that this interaction might be at the level of RPA, which helps mediate the switch to HR. To test this hypothesis, epitope-tagged Rad51p, Rad52p, or RPA were monitored for their ability to interact with Flag-tagged Mph1p. The immunoprecipitated Flag-Mph1p coprecipitated RPA but did not pull down either Rad51p or Rad52p (Fig. 4 C, top; and not depicted). Additionally, in the reverse immunoprecipitation, RPA-GFP pulled down Flag-Mph1p (Fig. 4 C, bottom). Mph1-CΔ protein did not interact with RPA (Fig. 4 C), which suggests that the loss of the

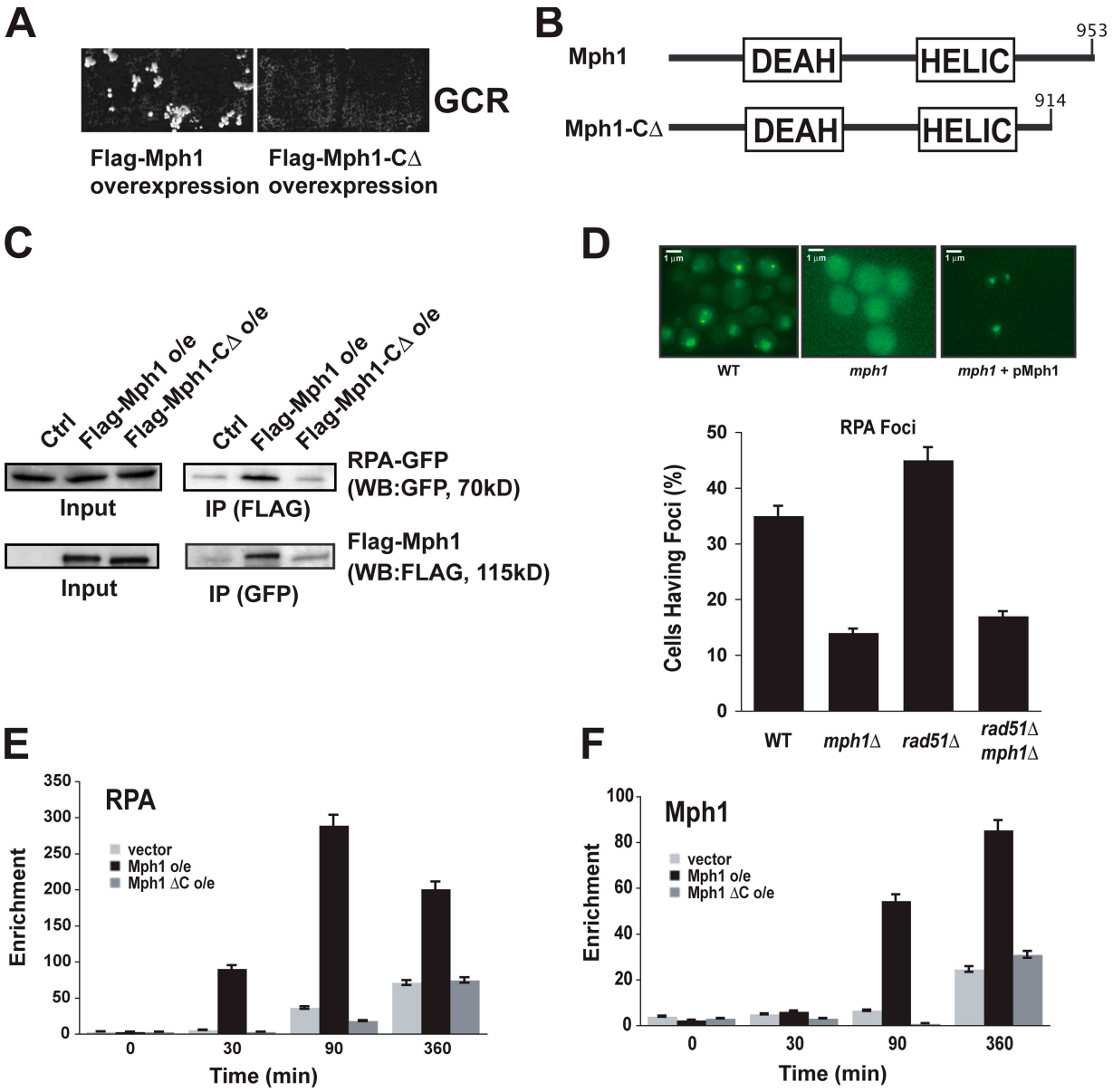

Figure 4. **The C-terminal motif of Mph1p for interaction with RPA has a critical role for GCR-promoting activity.** (A) Patch test of an *mph1Δ* mutation that no longer produced colonies resistant to canavanine and 5-FOA that reflected the absence of GCR. (B) Schematic demonstration of a mutation that did not show GCR enhancement when it was overexpressed. It was named Mph1-CΔ because it translates C-terminus–truncated Mph1p. (C) Mph1p interacts with RPA through its C-terminal motif. Immunoprecipitation of Mph1p through its Flag tag pulled down RPA that was detected by GFP tag at its C terminus (top) using ATCC201388 with different plasmids (MATa *his3Δ1leu2Δ0 met15Δ0 ura3Δ0 RFA1-GFP*). Immunoprecipitation of RPA pulled down the full-length Mph1p (bottom). Ctrl, control plasmid; o/e, overexpression; WT wild-type plasmid. (D) The *mph1Δ* mutation reduced the number of cells with spontaneous RPA foci that are independent of Rad51p. (top) Examples of GFP-RPA cells, ATCC201388: wild type, *mph1Δ*, and *mph1Δ* strain complemented by a plasmid expressing Mph1 (*mph1* + pMph1). (bottom) A graphic presentation of percentage of cells having spontaneous RPA foci from 100 cells from each strain counted. (E) Excess Mph1p enhanced RPA accumulation to DSB. ChIP of RPA at DSB with α-Rpa1p antibody was performed as described in Materials and methods. (F) Mph1p accumulated at DSB. ChIP of Mph1p was performed with α-HA antibody that recognizes the tag of Mph1p. Error bars represent standard deviation.

C terminus disrupted the interaction between RPA and Mph1. Thus, GCRs enhanced by excess Mph1p may be caused by a physical interaction between Mph1's C terminus and RPA. This interaction could compromise the role of RPA in DNA repair.

The reduction of Mph1p-induced GCRs by Rad52p overexpression could be caused by its competition with Mph1p for RPA interaction. To test this, we examined interactions under competitive conditions. The Mph1p–RPA interaction was not perturbed when Rad52p was overexpressed (Fig. S1 A, available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). In addition, MMS-induced Rad52p foci were not affected by either Mph1p or Mph1-ΔC overexpression (Fig. S1 B). Therefore, the reduction of Mph1p-induced GCRs by Rad52p overexpression is not caused by a direct competition with Mph1p for RPA interaction.

DNA damage during DNA replication produces long RPA-coated single-stranded DNA that is visualized as foci in the nucleus. The interaction between Mph1p and RPA suggests that Mph1p could affect loading and/or stabilizing RPA

on single-stranded DNA. To address this question, RPA foci formation was monitored in the *mph1Δ* strain expressing GFP-tagged Rpa1p. Even though we found no noticeable change in any phases of the cell cycle (not depicted), the number of cells with spontaneous RPA foci was significantly reduced in the *mph1Δ* strain (Fig. 4 D). Reintroduction of Mph1 via a single copy expression vector recovered RPA foci formation (Fig. 4 D, *mph1* + pMph1). Therefore, it is likely that Mph1p stabilizes RPA foci formation. Alternatively, fewer cells with RPA foci in *mph1Δ* could be caused by faster RPA turnover by efficient HR. Contrary to this idea, the loss of Rad51p did not restore the RPA foci levels in the *mph1Δ* strain (Fig. 4 D), and there was no noticeable change in HR rate in the *mph1Δ* strain (not depicted).

Slow recruitment of Rad51p to DSBs by excess Mph1p (Fig. 2 D) and fewer cells with RPA foci in the *mph1Δ* strain (Fig. 4 D) suggest that Mph1p could stabilize RPA at DNA damage. To test this hypothesis, RPA accumulation at DSB was monitored by ChIP with α-RPA antibody. Consistently, excess Mph1p enhanced the accumulation of RPA at DSB compared with controls (Fig. 4 E). In contrast, excess Mph1-CΔ could not enhance the accumulation of RPA. Lastly, we tested whether Mph1p is recruited to DSBs to stabilize RPA. ChIP analysis with an α-HA antibody that recognizes the tag of Mph1p demonstrated the enrichment of Mph1p, but not Mph1-CΔ, at the DSB (Fig. 4 F). Therefore, Mph1p seems to interact with and stabilize RPA at the site of DNA damage.

To determine whether there is any genetic interaction between *MPH1* and *RPA*, the effect of *mph1Δ* was examined when one of three MMS-sensitive alleles of the Rpa1 subunit of RPA (encoded by *RFA1*) were expressed (Figs. S2 and S3, available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). The *rfa1-t33* did not show any genetic interaction with the *mph1Δ* or Mph1p overexpression. The *rfa1-t11* mutation showed synergistic sensitivity to MMS only with the *mph1Δ* mutation (Fig. S2). The *rfa1-t48* mutation showed a partial rescue of MMS sensitivity by excess Mph1p (Fig. S3). Therefore, there are clear genetic interactions between *MPH1* and *RPA*.

Discussion

DNA damage could be repaired correctly or sometimes misrepaired to produce GCRs. Because of the complexity for choice of pathways to deal with DNA damage, cells need to have mechanisms to promote the most appropriate repair pathway. Our results suggest that Mph1p can promote a GCR pathway by partially suppressing HR.

Mph1p enhances GCRs by partially compromising HR and activating a GCR pathway (Figs. 1 and 2). The suppression of HR by Mph1p is likely achieved by stabilizing RPA (Fig. 4 E) binding of DNA, thereby blocking Rad52p-mediated Rad51p nucleofilament formation (Fig. 2 D). Consistent with these ideas, complete HR inactivation allowed excess Mph1p to promote GCR more efficiently (Fig. 1, B and C). The observation that Rad52p overexpression, but not that of Rad51p and Rad54p, could reduce Mph1p-induced GCRs (Fig. 2 C and not depicted) also suggests that Mph1p suppresses HR before Rad51p recruitment to the DSB. Notably, Mph1p was captured by affinity capture mass spectrometry using RPA as bait (Gavin et al., 2006), and physically interacted with RPA (Fig. 4 C). Collectively, we propose that Mph1p interacts with and stabilizes RPA-coated single-stranded DNA, and this prevents Rad52p-mediated Rad51p nucleofilament formation. This role of Mph1p is further supported by the observation that the *mph1Δ* mutation reduces the number of cells producing spontaneous or DNA damage–induced RPA foci (Fig. 4 D and not depicted).

Alternatively, it is possible that excess Mph1p could interfere with RPA or Rad51p-Rad52p recruitment to DNA damage by scavenging them. Nevertheless, there were no physical interactions between Mph1p and Rad52p or between Mph1p and Rad51p (unpublished data). Therefore, GCRs promoted by Mph1p are likely caused by the blocking of Rad51p-Rad52p through its interaction with RPA.

GCRs enhanced by excess Mph1p could be driven by the interference of DNA replication through its interaction with RPA. Even though excess Mph1p did not cause a significant change in the proportion of cells in S phase (unpublished data), we cannot rule out the possibility that the Mph1p-induced GCR enhancement arises when excess Mph1p perturbs DNA replication in the small proportion of cells that are not detectable by FACS analysis.

The GCR-promoting activity by Mph1p is required for GCRs produced in strains having *rad5Δ*, *rad18Δ*, *mec1Δ*, or *rfa1-t33* mutations under physiological conditions (Table I). For its GCR-promoting activity, Mph1p's interaction with RPA seems to be essential; in contrast, the helicase activity of Mph1p is only partially required (Table II). The blocking of Rad51p filament formation by Mph1p is solely dependent on its interaction with RPA, not its helicase activity (Figs. 3 and 4). Finally, because the motif mutants of Mph1 could still rescue the MMS sensitivity of *mph1Δ* (Fig. 3 D), only the loss of Mph1's GCR-promoting activity (i.e., its interaction with RPA) results in the synergistic sensitivity to MMS with the *srs2Δ* mutation. Thus, the RPA interaction seems to be essential for both the GCR-promoting activity and the Srs2p-like repair functions of Mph1p.

Even though excess Mph1p increased GCRs by partially suppressing HR, the complete inactivation of HR does not increase GCR when Mph1p is expressed in physiological conditions, except the *rad52Δ* mutation that also inactivates the break-induced replication that is important to suppress GCRs (Myung et al., 2001a). Therefore, partial HR activity is necessary to promote GCR, at least when Mph1p is expressed in physiological conditions. The requirement of partial HR activity for GCR formation is further supported by the suppression of GCRs in the *rad5Δ* or *rad18Δ* strain by the inactivation of HR (Motegi et al., 2006). This partial HR activity could be required to process DNA damage to produce intermediates, presumably DSB, for GCR formation. However, such activity might not be required if excess Mph1p covers RPA-coated single-stranded DNA and causes a break in the DNA. Alternatively, partial HR activity might allow GCR machinery to access DNA damage, whereas excess Mph1p could simply overcome such a requirement by blocking the access of other repair proteins.

One unique feature of Mph1p discovered in this study is the demonstration of its role in suppressing HR. Even though

there are several studies that suggest that *mph1Δ* is epistatic to mutations in HR genes (Scheller et al., 2000; Prakash et al., 2005; Onge et al., 2007), the *mph1Δ* mutation did not change the HR rate (unpublished data). No change in the HR rate by the *mph1Δ* mutation could be caused by the activation of postreplication repair by the *mph1Δ* mutation (Scheller et al., 2000). Elevated postreplication repair could bypass damaged DNA before HR repairs it in the *mph1Δ* strain, resulting in no change of the HR rate. Alternatively, Srs2p could suppress HR in the absence of Mph1p, which is supported by synergistic sensitivity to MMS by *mph1Δ* and *srs2Δ* mutation (Fig. 2 F).

Even though Srs2p could function similarly to Mph1p to promote GCR (Motegi et al., 2006), we did not detect GCR enhancement under the same expression system with Srs2p (unpublished data). This may be caused by the toxicity of Srs2p overexpression, which has been observed in a yeast Srs2p purification study (Krejci et al., 2003).

When cells reach late S or G2 phase, telomerase activity is high to replicate the end of chromosome (Marcand et al., 2000). This telomerase activity seems to promote de novo telomere addition–type GCRs. Excess Mph1p could augment GCRs from DNA damage by partially suppressing HR at the stalled replication forks. This sustained replication stall may lead to DSBs, thus providing substrates for active telomerase to carry out de novo telomere addition (the major type of GCR observed in this study).

Multiple choices to repair DNA lesions during DNA replication could result in different outcomes. Usually, these outcomes are beneficial for cells, but sometimes they can result in harmful mutations. In the present study, we uncovered Mph1p as an important decision maker between HR and GCR. The abnormal expression or mutation of *MPH1* can lead to undesirable outcomes, like GCRs (Mph1p overexpression) or mutations (*mph1Δ*; Scheller et al., 2000). Mph1p's putative human homologue FANCM could have a similar function for directing different DNA repair pathways. Therefore, the cancer predisposition observed in FA patients could be caused by erroneous repair choice.

Materials and methods

Yeast strains

S. cerevisiae strains used in this study for GCR, ChIP, and mating type switch; spontaneous recombination assay; and RPA interaction and RPA or Rad51p foci assays were isogenic to the S288c background strains RDKY3615 (*MATa, ura3-52, leu2Δ1, trp1Δ63, his3Δ200, lys2-Bgl, hom3-10, ade2Δ1, ade8, hxt13::URA3*), JKM161 (*Δho HMLalpha MATa Δhmr:; ADE1 ade1–100 leu2–3,112 lys5 trp1::hisg ura3–52 ade3::GAL-HO endonuclease his-*), M137-11B (*MATa can1-100 his3p::INV leu2 lys2-128 trp1 ura3*), and ATCC201388 (*MATa his3Δ1leu2Δ0 met15Δ0 ura3Δ0*), respectively.

General genetic methods

Conventional PCR-based gene disruption and plasmid transformation were used to generate strains. Yeast transformations were performed as described previously (Myung et al., 2001c; Smith et al., 2004). Relevant genotypes and plasmids are described in Table S4 (available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1). Yeast extract peptone-dextrose (YPD) and synthetic dropout media for propagating yeast strains and 5-fluoroorotic acid (5-FOA)–canavanine plates containing both 5-FOA and canavanine for selection of clones with GCR were prepared as described previously (Myung et al., 2001c; Smith et al., 2004). Previously, global genome-wide study showed synthetic lethality between the *mph1Δ* and *srs2Δ* mutations (Tong et al., 2004; Xu et al., 2004). However, the three different S288c background strains that we used did not show synthetic lethality. Strains carrying both mutations in this background showed a slight growth defect.

GCR rates and determination of rearrangement break point

All GCR rates were determined by fluctuation analysis using the method of the median with at least two independent clones (Lea and Coulson, 1948). The mean GCR rates from at least two or more independent experiments using either 5 or 11 cultures for each clone are reported as described previously (Myung et al., 2001c; Smith et al., 2004). The rearrangement breakpoints from mutants carrying an independent rearrangement were determined and classified as described previously (Myung et al., 2001c; Smith et al., 2004).

ChIP

The ChIP assay was performed as described previously (Shim et al., 2005), with some modifications. DSB of the log phase cells were induced by *HO* endonuclease by the addition of galactose to a final concentration of 2% (wt/vol). The expression of *HO* endonuclease was then repressed by the addition of glucose (2% final concentration) after 1 h. Cells collected at each time point were then cross-linked with 1% formaldehyde for 30 min. Cells were then washed and resuspended in 5 ml of spheroplast buffer (18.2% sorbitol, 1% glucose, 0.2% yeast nitrogen base, 0.2% casamino acids, 25 mM Hepes, pH 7.4, 50 mM Tris, and 1 mM DTT) with lyticase (4,000 units) and incubated for 30 min at 37°C to generate spheroplasts. After washing with ice-cold PBS buffer, Hepes/Triton X-100 buffer (0.25% Triton X-100, 10 mM EDTA, 0.5 mM EGTA, 10 mM Hepes, pH 6.5, 0.5 mM PMSF, 1 µg/ml pepstatin, and 1 µg/ml leupeptin), and Hepes/NaCl buffer (200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10 mM Hepes, pH 6.5, 0.5 mM PMSF, 1 µg/ml pepstatin, and 1 µg/ml leupeptin), spheroplasts were resuspended in 250 µl of SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1, 0.5 mM PMSF, 1 µg/ml pepstatin, and 1 µg/ml leupeptin) and sonicated to generate a mean DNA size of 0.5–1 kb. Supernatant after centrifugation was added into 2.5 ml of immunoprecipitation (IP) dilution buffer (1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris, pH 8.1, 167 mM NaCl, 0.5 mM PMSF, 1 µg/ml pepstatin, and 1 µg/ml leupeptin). IP was performed with 1 µl of anti-Rad51p, Rpa1p, or HA antibodies (1:5 dilution for Rad51p, provided by J. Haber [Brandeis University, Waltham, MA] and P. Sung [Yale University, New Haven, CT], 1:1 dilution for Rpa1p, provided by G. Brush [Wayne State University, Detroit, MI], 1:1 dilution for HA antibody), and coimmunoprecipitated DNA was amplified with primers that could bind near the MAT locus (5′-TCCCCATCGTCTTGCTCT-3′ and 5′-GCATGGGCAGTTTACCTTTAC-3′) and primers that amplify the *ACT1* locus for control (5′-CCAATTGCTCGAGAGATTTC-3′ and 5′-CATGATACCTTGGTGTCTTG-3′). All samples were quantified by real-time PCR (7500 Real Time PCR system; Applied Biosystems). PCR was performed in 25-µl reactions with 1/46 of the immunoprecipitates and 1/2,000 of input DNA, 200 nM per primer, and platinum SYBR green qPCR SuperMix-UDG (Invitrogen). PCR cycling was conducted at 50°C for 2 min and 95°C for 2 min followed by 40 cycles at 95°C for 15 s, 55°C for 30 s, and 72°C for 35 s. The relative proportions of ChIPed DNA fragments were calculated by the formula $2^{Ct(input)}/2^{Ct(IP)}$. Ct(input) and Ct(IP) are the threshold cycle (Ct) values from each input sample and from each IP sample, respectively.

Random mutagenesis

XL1-Red competent cells (Stratagene) were used for random mutagenesis of *MPH1* according to the manufacturer's instructions. pKJM528 was transformed into XL1-Red cells, and the pools of transformed colonies were cultured overnight. Plasmids isolated from bacteria grown overnight were directly transformed into RDKY3615, and colonies resistant to G418 were collected. One patch (1 × 1 cm in size) for each colony was grown at 30°C for 2 d and replica plated onto a 5-FOA–canavanine plate. After a 3-d incubation at 30°C, patches that either had zero or a reduced number of resistant colonies (representing lower GCR) were selected. Plasmids were isolated from the original colony whose patch showed reduction in GCR and were amplified in *E. coli*, *DH5α*. Plasmids recovered from three independent bacterial colonies were retransformed into RDKY3615, and GCR reduction was confirmed by patch testing. Plasmids were then sequenced to find mutations that caused defects in GCR enhancement.

Mating type switching assay and spontaneous recombination assay

Homologous recombination efficiency measured by mating type switching was performed as described previously (Wu et al., 1997) with control or Mph1p overexpression plasmids. Spontaneous recombination rates were

measured as described previously (Aguilera and Klein, 1989) with control or Mph1p overexpression plasmids or with the mutations described.

Sensitivity to DNA-damaging agents

To detect MMS sensitivity in chronic exposure, cells in exponential phase were serially diluted, and 5 µl of the cells were spotted on YPD plates and YPD plates with the indicated dose of MMS. To detect γ-ray sensitivity, one YPD plate spotted with cells was irradiated with the indicated doses of γ-ray radiation. After 2–3 d of incubation at 30°C, pictures were taken. To determine MMS sensitivity in acute exposure for Fig. 3 D, cells in exponential phase were treated in indicated dose of MMS for 2 h, and surviving cells were determined by plating on YPD plates after serial dilution. Each colony number from a different dose of MMS treatment was normalized by setting the number of colonies with no treatment as 100%.

RPA foci formation

The RPA foci formation assay was performed as described previously (Lisby et al., 2004). In brief, cells were grown in 2-ml YPD media at 30°C for overnight. 400 µl of cell suspension was taken and grown in 10 ml synthetic dropout media at 25°C in dark conditions. After 4 h, when cells were in log phase, cells were diluted in water (1:20 dilution) and washed with water three times. Cells were further incubated at 30°C with nuclear-staining Hoechst dye for 10 min and harvested. Cells were then resuspended in 2–3 µl of water and placed on the glass slide covered with a glass coverslip. The images were acquired using a DeltaVision Personal live cell system (Applied Precision, LLC) mounted on an inverted microscope (IX-71; Olympus) with a UPlan-SApo 100× 1.4 NA oil immersion objective (Applied Precision, LLC). Each z-stacked image (five optical images) was captured using a CoolSnap ES2 camera (Applied Precision, LLC) with a 0.3-µm z interval. GFP-positive cells were acquired using a 528/38-nm emission filter, CFP positive cells were acquired using a 470/30-nm emission filter, and the Hoechst was collected using a 457/50-nm emission filter. All image sets were first deconvolved using Applied Precision's restoration 3D algorithm (nonsubtractive method) in SoftWoRx version 3.6.2, then loaded into the Imaris 3D software package (version 5.7; Bitplane) for volume rendering and spot recognition.

Online supplemental material

Fig. S1 shows that the reduced GCR by Rad52p cooverexpression was not caused by a direct competition of Rad52p for Mph1p interaction with RPA. Fig. S2 shows genetic interactions between different *rfa1* alleles and *mph1Δ*. Fig. S3 shows genetic interactions between different *rfa1* alleles and Mph1p overexpression. Table S1 shows the actual GCR rates presented in Fig. 1. Table S2 demonstrates the reduced long track with crossover gene conversion by excess Mph1p. Table S3 shows the actual GCR rates presented in Fig. 3 B. Table S4 shows genotypes of strains used in this study. Online supplemental material is available at http://www.jcb.org/cgi/content/full/jcb.200711146/DC1.

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References

Aguilera, A., and H.L. Klein. 1989. Yeast intrachromosomal recombination: long gene conversion tracts are preferentially associated with reciprocal exchange and require the RAD1 and RAD3 gene products. *Genetics.* 123:683–694.

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.

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.

Cheok, C.F., C.Z. Bachrati, K.L. Chan, C. Ralf, L. Wu, and I.D. Hickson. 2005. Roles of the Bloom's syndrome helicase in the maintenance of genome stability. *Biochem. Soc. Trans.* 33:1456–1459.

Clarke, L., and J. Carbon. 1976. A colony bank containing synthetic Col E1 hybrid plasmids representative of the entire *E. coli* genome. *Cell.* 9:91–99.

Firmenich, A.A., M. Elias-Arnanz, and P. Berg. 1995. A novel allele of *Saccharomyces cerevisiae* RFA1 that is deficient in recombination and repair and suppressible by RAD52. *Mol. Cell. Biol.* 15:1620–1631.

Gari, K., C. Decaillet, A.Z. Stasiak, A. Stasiak, and A. Constantinou. 2008. The Fanconi anemia protein FANCM can promote branch migration of Holliday junctions and replication forks. *Mol. Cell.* 29:141–148.

Gavin, A.C., P. Aloy, P. Grandi, R. Krause, M. Boesche, M. Marzioch, C. Rau, L.J. Jensen, S. Bastuck, B. Dumpelfeld, et al. 2006. Proteome survey reveals modularity of the yeast cell machinery. *Nature.* 440:631–636.

Hwang, J.Y., S. Smith, and K. Myung. 2005. The Rad1-Rad10 complex promotes the production of gross chromosomal rearrangements from spontaneous DNA damage in *Saccharomyces cerevisiae. Genetics.* 169:1927–1937.

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.

Kennedy, R.D., and A.D. D'Andrea. 2005. The Fanconi Anemia/BRCA pathway: new faces in the crowd. *Genes Dev.* 19:2925–2940.

Kolodner, R.D., C.D. Putnam, and K. Myung. 2002. Maintenance of genome stability in *Saccharomyces cerevisiae. Science.* 297:552–557.

Komori, K., M. Hidaka, T. Horiuchi, R. Fujikane, H. Shinagawa, and Y. Ishino. 2004. Cooperation of the N-terminal Helicase and C-terminal endonuclease activities of Archaeal Hef protein in processing stalled replication forks. *J. Biol. Chem.* 279:53175–53185.

Krejci, L., S. Van Komen, Y. Li, J. Villemain, M.S. Reddy, H. Klein, T. Ellenberger, and P. Sung. 2003. DNA helicase Srs2 disrupts the Rad51 presynaptic filament. *Nature.* 423:305–309.

Lea, D.E., and C.A. Coulson. 1948. The distribution of the numbers of mutants in bacterial populations. *J. Genet.* 49:264–285.

Lengauer, C. 2005. Aneuploidy and genetic instability in cancer. *Semin. Cancer Biol.* 15:1.

Lengronne, A., and E. Schwob. 2002. The yeast CDK inhibitor Sic1 prevents genomic instability by promoting replication origin licensing in late G(1). *Mol. Cell.* 9:1067–1078.

Levran, O., C. Attwooll, R.T. Henry, K.L. Milton, K. Neveling, P. Rio, S.D. Batish, R. Kalb, E. Velleuer, S. Barral, et al. 2005. The BRCA1-interacting helicase BRIP1 is deficient in Fanconi anemia. *Nat. Genet.* 37:931–933.

Lisby, M., J.H. Barlow, R.C. Burgess, and R. Rothstein. 2004. Choreography of the DNA damage response: spatiotemporal relationships among checkpoint and repair proteins. *Cell.* 118:699–713.

Marcand, S., V. Brevet, C. Mann, and E. Gilson. 2000. Cell cycle restriction of telomere elongation. *Curr. Biol.* 10:487–490.

Meetei, A.R., A.L. Medhurst, C. Ling, Y. Xue, T.R. Singh, P. Bier, J. Steltenpool, S. Stone, I. Dokal, C.G. Mathew, et al. 2005. A human ortholog of archaeal DNA repair protein Hef is defective in Fanconi anemia complementation group M. *Nat. Genet.* 37:958–963.

Mosedale, G., W. Niedzwiedz, A. Alpi, F. Perrina, J.B. Pereira-Leal, M. Johnson, F. Langevin, P. Pace, and K.J. Patel. 2005. The vertebrate Hef ortholog is a component of the Fanconi anemia tumor-suppressor pathway. *Nat. Struct. Mol. Biol.* 12:763–771.

Motegi, A., and K. Myung. 2007. Measuring the rate of gross chromosomal rearrangements in *Saccharomyces cerevisiae*: A practical approach to study genomic rearrangements observed in cancer. *Methods.* 41:168–176.

Motegi, A., K. Kuntz, A. Majeed, S. Smith, and K. Myung. 2006. Regulation of gross chromosomal rearrangements by ubiquitin and SUMO ligases in *Saccharomyces cerevisiae. Mol. Cell. Biol.* 26:1424–1433.

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.

Onge, R.P., R. Mani, J. Oh, M. Proctor, E. Fung, R.W. Davis, C. Nislow, F.P. Roth, and G. Giaever. 2007. Systematic pathway analysis using high-resolution fitness profiling of combinatorial gene deletions. *Nat. Genet.* 39:199–206.

Opresko, P.L., W.H. Cheng, and V.A. Bohr. 2004. Junction of RecQ helicase biochemistry and human disease. *J. Biol. Chem.* 279:18099–18102.

Prakash, R., L. Krejci, S. Van Komen, K. Anke Schurer, W. Kramer, and P. Sung. 2005. *Saccharomyces cerevisiae* MPH1 gene, required for homologous recombination-mediated mutation avoidance, encodes a 3′ to 5′ DNA helicase. *J. Biol. Chem.* 280:7854–7860.

Scheller, J., A. Schurer, C. Rudolph, S. Hettwer, and W. Kramer. 2000. MPH1, a yeast gene encoding a DEAH protein, plays a role in protection of the genome from spontaneous and chemically induced damage. *Genetics.* 155:1069–1081.

Schmidt, K.H., J. Wu, and R.D. Kolodner. 2006. Control of translocations between highly diverged genes by Sgs1, the *Saccharomyces cerevisiae* homolog of the Bloom's syndrome protein. *Mol. Cell. Biol.* 26:5406–5420.

Schulz, V.P., and V.A. Zakian. 1994. The *Saccharomyces* PIF1 DNA helicase inhibits telomere elongation and de novo telomere formation. *Cell.* 76:145–155.

Schurer, K.A., C. Rudolph, H.D. Ulrich, and W. Kramer. 2004. Yeast MPH1 gene functions in an error-free DNA damage bypass pathway that requires genes from homologous recombination, but not from postreplicative repair. *Genetics.* 166:1673–1686.

Shim, E.Y., J.L. Ma, J.H. Oum, Y. Yanez, and S.E. Lee. 2005. The yeast chromatin remodeler RSC complex facilitates end joining repair of DNA double-strand breaks. *Mol. Cell. Biol.* 25:3934–3944.

Smith, S., J.-Y. Hwang, S. Banerjee, A. Majeed, A. Gupta, and K. Myung. 2004. Mutaor genes for suppression of gross chromosomal rearrangements identified by a genome-wide screening in *Saccharoymces cerevisiae. Proc. Natl. Acad. Sci. USA.* 101:9039–9044.

Symington, L.S. 2002. Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. *Microbiol. Mol. Biol. Rev.* 66:630–670.

Tanaka, S., and J.F. Diffley. 2002. Deregulated G1-cyclin expression induces genomic instability by preventing efficient pre-RC formation. *Genes Dev.* 16:2639–2649.

Teixeira, M.R., and S. Heim. 2005. Multiple numerical chromosome aberrations in cancer: what are their causes and what are their consequences? *Semin. Cancer Biol.* 15:3–12.

Tong, A.H., G. Lesage, G.D. Bader, H. Ding, H. Xu, X. Xin, J. Young, G.F. Berriz, R.L. Brost, M. Chang, et al. 2004. Global mapping of the yeast genetic interaction network. *Science.* 303:808–813.

Veaute, X., J. Jeusset, C. Soustelle, S.C. Kowalczykowski, E. Le Cam, and F. Fabre. 2003. The Srs2 helicase prevents recombination by disrupting Rad51 nucleoprotein filaments. *Nature.* 423:309–312.

Wu, X., C. Wu, and J.E. Haber. 1997. Rules of donor preference in *Saccharomyces* mating-type gene switching revealed by a competition assay involving two types of recombination. *Genetics.* 147:399–407.

Xu, H., C. Boone, and H.L. Klein. 2004. Mrc1 is required for sister chromatid cohesion to aid in recombination repair of spontaneous damage. *Mol. Cell. Biol.* 24:7082–7090.

Zhou, J., E.K. Monson, S.C. Teng, V.P. Schulz, and V.A. Zakian. 2000. Pif1p helicase, a catalytic inhibitor of telomerase in yeast. *Science.* 289:771–774.