Journal of Microbiology (2013) Vol. 51, No. 1, pp. 105–112
DOI 10.1007/s12275-013-3048-2

Hrq1 Functions Independently of Sgs1 to Preserve Genome Integrity in *Saccharomyces cerevisiae*

Do-Hee Choi, Rina Lee, Sung-Hun Kwon, and Sung-Ho Bae*

Department of Biological Sciences, College of Natural Science, Inha University, Incheon 402-751, Republic of Korea

(Received January 21, 2013 / Accepted January 29, 2013)

Maintenance of genome stability in eukaryotes involves a number of conserved proteins, including RecQ helicases, which play multiple roles at various steps in homologous recombination and DNA repair pathways. Sgs1 has been described as the only RecQ helicase in lower eukaryotes. However, recent studies revealed the presence of a second RecQ helicase, Hrq1, which is most homologous to human RECQL4. Here we show that *hrq1Δ* mutation resulted in increased mitotic recombination and spontaneous mutation in *Saccharomyces cerevisiae*, and *sgs1Δ* mutation had additive effects on the phenotypes of *hrq1Δ*. We also observed that the *hrq1Δ* mutant was sensitive to 4-nitroquinoline 1-oxide and cisplatin, which was not complemented by overexpression of Sgs1. In addition, the *hrq1Δ sgs1Δ* double mutant displayed synthetic growth defect as well as a shortened chronological life span compared with the respective single mutants. Analysis of the type of age-dependent Can^r mutations revealed that only point mutations were found in *hrq1Δ*, whereas significant numbers of gross deletion mutations were found in *sgs1Δ*. Our results suggest that Hrq1 is involved in recombination and DNA repair pathways in *S. cerevisiae* independent of Sgs1.

Keywords: Hrq1, RecQ helicase, DNA helicase, RECQL4 orthologue, Sgs1, recombination

Introduction

Eukaryotic cells contain a number of conserved proteins involved in DNA repair pathways in order to maintain genome integrity against various kinds of exogenous and/or endogenous environmental threats, such as genotoxic agents and aberrant DNA structures generated during DNA metabolism. RecQ helicases are evolutionary conserved from bacteria to humans and play multiple roles at various steps in homologous recombination (HR) and DNA repair pathways (Chu and Hickson, 2009; Bernstein *et al*., 2010). Bacteria have only a single RecQ helicase, whereas human cells contain five RecQ proteins (RECQL1, BLM, WRN, RECQL4, and RECQL5). BLM and WRN are linked to autosomal recessive diseases, Bloom syndrome and Werner syndrome, respectively (Bohr, 2008; Monnat, 2010). Mutations in RECQL4 are responsible for three rare genetic disorders, Rothmund-Thomson syndrome (RTS), Baller-Geroid syndrome, and RAPADILINO syndrome. These heritable diseases are characterized by cancer predisposition and premature aging phenotypes resulting from increased genome instability (Bohr, 2008). In contrast, the remaining two helicases, RECQL1 and RECQL5, have not been implicated in any human disease.

Sgs1 and Rqh1, most homologous to human BLM, are members of the RecQ helicase family in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively, and have multiple roles in double strand break (DSB) repair by HR (Ashton and Hickson, 2010; Singh *et al*., 2012). Their homologies are not limited to amino acid sequence but extend to protein-protein interactions. BLM physically interacts with type IA topoisomerase, hTOPOIIIa, hRMI1, and hRMI2. Sgs1 and Rqh1 also form a similar complex with Top3 and Rmi1. This complex plays a role in the dissolution of double Holliday junctions to form exclusively non-crossover products (Ashton and Hickson, 2010). Sgs1 and Rqh1 also suppress illegitimate recombination by unwinding aberrantly paired DNA molecules at regions of limited homology (Chu and Hickson, 2009; Ashton and Hickson, 2010; Bernstein *et al*., 2010). This is consistent with the hyper-recombination phenotypes of *sgs1* mutants (Onoda *et al*., 2000, 2004a). Recent studies suggested that BLM and Sgs1 collaborate with Dna2 and exonuclease 1 for extensive 5′-end resection to form a 3′-single-stranded DNA (ssDNA) tail, a key early step in HR (Mimitou and Symington, 2008; Zhu *et al*., 2008). Since HR is the most important error-free mechanism of DSB repair, absence of BLM and Sgs1 function affects the initiation of DSB repair by HR and hence elevates genome instabilities in human and *S. cerevisiae*, respectively (Singh *et al*., 2012). BLM and Sgs1 also play important roles in DNA replication to maintain genome stability. They are required for stabilization of polymerases at stalled replication forks and HR-mediated replication restart (Cobb *et al*., 2005; Davies *et al*., 2007; Wu, 2007).

Until recently, Sgs1 and Rqh1 were considered as the only RecQ orthologues in *S. cerevisiae* and *S. pombe*, respectively (Ashton and Hickson, 2010; Bernstein *et al*., 2010). Although *S. pombe* contains other RecQ-like proteins (Tlh1 and Tlh2), they appear to participate in telomere metabolism only during crisis of telomere erosion (Mandell *et al*., 2005). However, another highly conserved RecQ-homologous protein, Hrq1, has been found in fungal genomes by bioinformatics analyses and is most similar to human RECQL4 (Barea *et al*., 2008).

*For correspondence. E-mail: sbae@inha.ac.kr; Tel.: +82-32-860-7712; Fax: +82-32-874-6737

Table 1. Yeast strains used in this study

Strain	Genotype	Reference
YPH499	*MAT a ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	Sikorski and Hieter (1989)
YSH40	*MAT a ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hrq1Δ::HIS3*	This study
YDH920	*MAT a ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgs1Δ::HIS3*	This study
YSH45	*MAT a ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hrq1Δ::TRP1 sgs1Δ::HIS3*	This study
SCRMTL2	*MAT a ade2 ade3-130 leu2 trp1 ura3 cyh2 SCR::LEU2*	Onoda *et al.* (2004b)
YSH79	*MAT a ade2 ade3-130 leu2 trp1 ura3 cyh2 SCR::LEU2 hrq1Δ::TRP1*	This study
YLN8	*MAT a ade2 ade3-130 leu2 trp1 ura3 cyh2 SCR::LEU2 sgs1Δ::URA3*	This study
YLN17	*MAT a ade2 ade3-130 leu2 trp1 ura3 cyh2 SCR::LEU2 hrq1Δ::TRP1 sgs1Δ::URA3*	This study
MR101	*MAT a/α ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289 his1-7/his1-1*	Resnick *et al.* (1984)
YLN6	*MAT a/α ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289 his1-7/his1-1 hrq1Δ::TRP1/hrq1Δ::URA3*	This study
YLN5	*MAT a/α ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289 his1-7/his1-1 sgs1Δ::URA3/sgs1Δ::LEU2*	This study
YLN7	*MAT a/α ura3-52/ura3-52 leu2-3,112/leu2-3,112 trp1-289/trp1-289 his1-7/his1-1 hrq1Δ::TRP1/hrq1Δ::URA3 sgs1Δ::URA3/sgs1Δ::LEU2*	This study

Recent genetic studies have shown that Hrq1 is a functional gene in *S. pombe* (Groocock *et al.*, 2012). Hrq1-deficient cells suffer spontaneous genome instability and exhibit hyper-recombination and mutator phenotypes. Genetic epistasis analysis suggested that Hrq1 is involved in nucleotide excision repair (NER) pathway (Groocock *et al.*, 2012). Very recently, we have reported the biochemical properties of *S. cerevisiae* Hrq1 protein (Kwon *et al.*, 2012). Purified recombinant Hrq1 protein exhibits moderately processive 3′-5′ helicase activity and requires a long 3′-ssDNA tail for efficient unwinding of duplex DNA. It also has potent DNA strand annealing activity similar to other RecQ helicases, suggesting that Hrq1 is another conserved RecQ helicase in yeast.

Here we attempted to gain insights into the *in vivo* functions of Hrq1 in *S. cerevisiae* and determine whether Hrq1 and Sgs1 have overlapping roles in DNA metabolism. For this purpose, we constructed *hrq1Δ* and *sgs1Δ* mutations and investigated their effects on recombination and DNA repair. In this report, we demonstrate that Hrq1 and Sgs1 function in parallel pathways for maintenance of genome stability. We also show that Hrq1 is important in regulating chronological life span (CLS) of yeast.

Materials and Methods

Yeast strains and plasmids

All *S. cerevisiae* strains used in this study are listed in Table 1. The yeast media, YPD and synthetic drop-out (SD), were prepared as described (Chris *et al.*, 1994). Gene disruptions were conducted by one-step gene replacement methods as previously described (Sikorski and Hieter, 1989; Baudin *et al.*, 1993). In order to disrupt *HRQ1* and *SGS1*, selective markers were PCR-amplified using primers harboring 55 base-pairs (bp) homologous to the 5′ or 3′ flanking region of each open reading frame (ORF), and transformed into yeast cells to replace the target genes. Yeast colonies that grew on selective medium were analyzed by PCR for correct disruption. To construct diploid gene disruptants, MATa and MATα haploid gene disruptants were made and mated.

To construct a single copy plasmid containing a constitutive promoter, the *ADH1* promoter region was PCR-amplified and cloned into the *Sac*I-*Not*I sites of pRS315 (New England Biolabs, USA). The resulting plasmid was named pRS315-*ADH1*. Then, the entire *HRQ1* ORF was PCR-amplified and cloned into the *Pst*I-*Xho*I sites of pRS315-*ADH1* in order to obtain pRS315-*ADH1-HRQ1*, which expresses Hrq1 protein under the control of *ADH1* promoter. The multi-copy plasmid pRS325-*ADH1-HRQ1* was constructed by subcloning the *Sac*I-*Xho*I fragment from pRS315-*ADH1-HRQ1* into pRS325. The *hrq1K318A* mutant gene, containing a base substitution mutation in the ATP-binding motif of Hrq1 protein, was described previously (Kwon *et al.*, 2012). The multi-copy plasmid pRS325-*ADH1-SGS1* was constructed by cloning the PCR-amplified *SGS1* ORF into the *Bam*HI-*Sal*I sites of pRS325-*ADH1*.

Measurement of recombination and spontaneous mutation frequencies

The strains derived from SCRMTL2 (Onoda *et al.*, 2004b) were used to measure the frequencies of unequal sister chromatid recombination (USCR). These strains contain two truncated *ADE3* genes, 5′ and 3′ deletions. These two partial genes have a short overlap, and reciprocal recombination or gene conversion in this overlap results in restoration of functional *ADE3* gene (Kadyk and Hartwell, 1992). Since the product of *ADE3* participates in histidine prototropy, the recombination frequency can be monitored by counting colonies on SD plates lacking histidine. The strains derived from MR101 (Resnick *et al.*, 1984) were used to measure inter-chromosomal recombination frequencies. These diploid strains contain hetero-alleles, his1-1/his1-7, and recombination between these two mutations allows the restoration of histidine prototropy (Onoda *et al.*, 2004b). To measure recombination frequencies, cells from saturated cultures were spread onto SD plates lacking histindine and YPD plates in order to determine recombination events and the total number of viable cells, respectively.

Spontaneous mutation frequencies were determined by counting *CAN1* mutations. *CAN1* gene encodes arginine permease, and mutation of this gene confers resistance to the

Table 2. Doubling time and recombination frequencies of *hrq1Δ* cells

Strain	Doubling time (min)	Recombination frequency ($\times 10^{-5}$)	
		Unequal sister chromatid exchange	Inter-chromosomal recombination
WT	121.8 ± 4.2[a]	2.3 ± 0.3[a]	1.7 ± 0.3[a]
hrq1Δ	139.8 ± 1.6	3.1 ± 1.0	1.6 ± 0.2
sgs1Δ	137.2 ± 1.4	6.4 ± 1.2	12.8 ± 4.0
hrq1Δ sgs1Δ	166.2 ± 7.8	11.0 ± 2.1	19.4 ± 10.4

[a] The averages and standard deviations from three independent experiments are presented.

arginine analogue canavanine, which makes it easy to detect *CAN1* mutations by selection of canavanine-resistant (Can^r) colonies. Cells were spread onto canavanine-containing (60 μg/ml) SD plates, and mutant colonies were counted after 3–4 days incubation at 30°C. Cell viability was measured by plating appropriate dilutions onto YPD plates and counting colony-forming units (CFUs).

Measurement of sensitivity to chemical agents and UV-irradiation

Yeast cells were grown to stationary phase in liquid SD media, diluted 1:1,000, and then grown to mid-log phase until all strains reached a similar OD_{600}. For exposure to DNA-damaging agents, 10-fold serial dilutions of cell culture from 10^7 cells/ml were spotted onto SD plates containing the selected chemicals. The plates were then incubated for 3–4 days at 30°C. Hydroxyurea (HU, 20–100 mM), methyl methanesulfonate (MMS, 0.01%), 4-nitroquinoline 1-oxide (4-NQO, 0.02–0.3 μg/ml), and cisplatin (20–50 μg/ml) were added to SD plates. For the UV sensitivity tests, SD plates spotted with yeast cells were irradiated with various UV dosages (40–100 J/m^2) and incubated in the dark at 30°C.

Measurement of CLS and age-dependent mutation frequency

Yeast cells were picked from fresh colonies and grown to stationary phase in liquid SD media. Cultures were diluted to OD 0.05 with SD media and incubated at 30°C with continuous shaking. Aliquots were removed at different time points, diluted appropriately, and spread onto YPD plates to count CFUs and canavanine-containing (60 μg/ml) SD plates to measure age-dependent spontaneous mutation frequency. The number of CFUs on day 1 was considered as the initial survival (100%), and the relative ratios of survival were plotted.

CAN1 sequencing

Can^r clones from wild-type and *hrq1Δ* strains were collected on day 7 of the CLS study. Genomic DNA was isolated using the standard glass beads/choloroform-phenol procedure, and the entire *CAN1* ORFs were PCR-amplified using the following primer set: CAN1-1 (5′-TAA TCT GTC GTC AAT CGA AAG-3′) and CAN1-2 (5′-GGG AGC AAG ATT GTT GTG GT-3′). The PCR products were gel-purified and sequenced using the amplification primers and the following additional primers: CAN1-3 (5′-TGC CTG GGG TCC AGG TAT AA-3′) and CAN1-4 (5′-ACG CAG TCC TTG GGT GAA ATG GC-3′).

Results

The *hrq1Δ sgs1Δ* double mutant displays synthetic growth defect and hyper-recombination phenotype

We recently reported that Hrq1 is an active RecQ helicase in *S. cerevisiae* (Kwon *et al.*, 2012). To gain further insights into the *in vivo* functions of Hrq1, we constructed various *hrq1Δ* mutant strains and investigated their phenotypic changes in terms of growth rates and frequencies of mitotic recombination (Table 2). First, we investigated growth of the mutant strains by estimating doubling time. Compared with the wild-type strain (YPH499), the single mutant strains *hrq1Δ* and *sgs1Δ* of the same genetic background showed slightly slower growth rates. On the other hand, the *hrq1Δ sgs1Δ* double mutant strain showed significant inhibition of growth (Table 2), indicating that the *hrq1Δ* and *sgs1Δ* mutations have additive effects on the growth of yeast cells. This result implies that the slower growth observed in individual mutant strains most likely arise from distinct defects.

The *sgs1Δ* and *rqh1Δ* (Sgs1 orthologue of *S. pombe*) mutants display hyper-recombination phenotype (Ashton and Hickson, 2010). This is most probably due to the anti-recombination activity of Sgs1, which resolves illegitimate recombination intermediates for suppression of aberrant HR. Recently, hyper-recombination phenotype was also observed in *S. pombe hrq1* mutant strains (Groocock *et al.*, 2012). Therefore, we investigated whether *hrq1* mutation also affects recombination frequency in *S. cerevisiae*. We first measured frequency of recombination between sister chromatids using tester strains constructed from SCRMTL2 (Onoda *et al.*, 2004b) as described in 'Materials and Methods'. This is based on the restoration of histidine prototrophy produced by reciprocal recombination or gene conversion (Kadyk and Hartwell, 1992). The *hrq1Δ* strain displayed a slightly elevated frequency of His$^+$ colony formation in comparison with wild-type, whereas the frequency was lower compared to the *sgs1Δ* strain (Table 2). While each individual mutation had only a marginal effect on USCR frequency, simultaneous deletion of *HRQ1* and *SGS1* resulted in a synergistic increase in USCR frequency compared with the respective single mutants (Table 2). To confirm these findings, we determined whether inter-chromosomal recombination frequencies are elevated in the *hrq1Δ sgs1Δ* double mutant using strains derived from diploid strain MR101. Recombination between *his1-1*/*his1-7* heteroalleles in this strain can be detected by the restoration of histidine prototrophy (Onoda *et al.*, 2004b). Consistent with previous observations (Watt *et al.*, 1996), the *sgs1Δ* mutant showed increased recombination frequency, whereas *hrq1Δ* mutation had no effect on the inter-chromosomal recombination frequency (Table 2). Nevertheless, additional

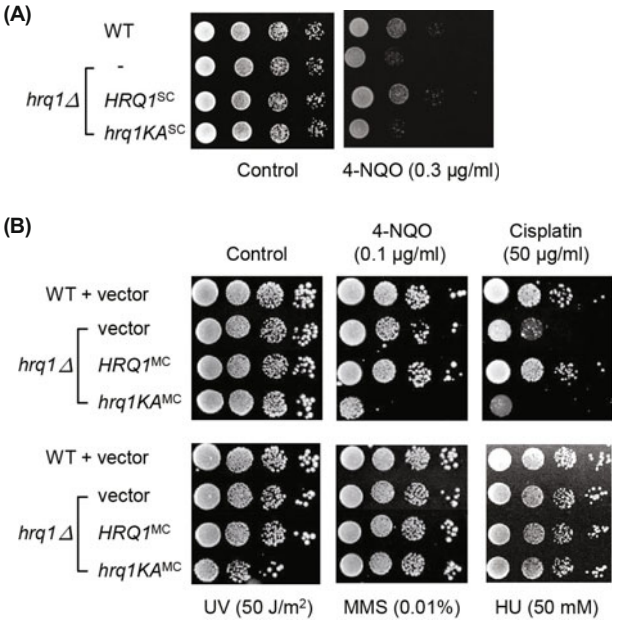

Fig. 1. Sensitivity of *hrq1Δ* mutant to DNA-damaging agents. (A) Strains YPH499 (WT) and YSH40 (*hrq1Δ*) were spotted in serial 10-fold dilutions onto SD media containing 4-NQO (0.3 μg/ml), followed by incubation for 3 days at 30°C. $HRQ1^{SC}$ and $hrq1KA^{SC}$ denote YSH40 transformed with single-copy plasmids pRS315-*ADH1-HRQ1* and pRS315-*ADH1-hrq1K318A*, respectively. (B) Dominant negative effects of *hrq1KA* mutant. Strains YPH499 (WT) and YSH40 (*hrq1Δ*) were transformed with multi-copy plasmids pRS325-*ADH1* (vector), pRS325-*ADH1-HRQ1* ($HRQ1^{MC}$), and pRS325-*ADH1-hrq1K318A* ($hrq1KA^{MC}$). Transformants were grown to saturation, and serial dilutions were spotted onto SD media containing 4-NQO (0.1 μg/ml), cisplatin (50 μg/ml), MMS (0.01%), and hydroxyurea (HU, 50 mM). To test UV sensitivity, cells spotted on SD media were irradiated with UV (50 J/m^2).

deletion of *HRQ1* in the *sgs1Δ* mutant resulted in further elevation of the recombination frequency, demonstrating that *hrq1Δ* and *sgs1Δ* mutations have additive effects on the hyper-recombination phenotype. If Hrq1 and Sgs1 proteins sequentially function in the same pathway, one mutation should be epistatic to the other. The synergistic effects of *hrq1Δ* and *sgs1Δ* rule out this possibility and imply that *HRQ1* and *SGS1* independently influence the recombination frequency.

The *hrq1Δ* mutant is sensitive to DNA damage induced by 4-NQO and cisplatin

We next examined the sensitivities of *hrq1Δ* mutant cells to various DNA-damaging agents. This was determined by UV-irradiation or continuous exposure to MMS, HU, cisplatin, and 4-NQO using drop-dilution assay. The *hrq1Δ* mutant was found to be slightly sensitive to 4-NQO (Fig. 1A) and cisplatin (Fig. 1B) but not to MMS, HU, or UV-irradiation (Fig. 1B). In addition, the growth defect of *hrq1Δ* mutant was rescued by the expression of wild-type *HRQ1* gene but not by the *hrq1K318A* helicase-deficient mutant gene (Fig. 1A), indicating that 4-NQO sensitivity is caused by a lack of Hrq1 helicase activity. Previously, we have shown that Hrq1K318A mutant protein is completely devoid of both ATPase and helicase activities (Kwon *et al.*, 2012).

The genotoxin sensitivity profile of *S. cerevisiae hrq1Δ* mutant is likely to be similar to that of *S. pombe* Hrq1 mutant cells. The *S. pombe hrq1Δ* mutant was hypersensitive to cisplatin but showed negligible sensitivity to UV-irradiation, HU, and MMS (Groocock *et al.*, 2012). In addition, *S. pombe* mutant cells containing the helicase-dead form of Hrq1, Hrq1-HD, were more sensitive to cisplatin than *hrq1Δ* cells, suggesting that Hrq1-HD dominantly interferes with other repair processes (Groocock *et al.*, 2012). However, the *hrq1-K318A* mutant gene expressed in the single-copy plasmid did not have any deleterious effect in our experiment (Fig. 1A). This prompted us to examine whether *hrq1K318A* in the multi-copy plasmid displayed dominant negative phenotypes. For this purpose, *hrq1Δ* mutant cells were transformed with multi-copy plasmids expressing either wild-type Hrq1 or Hrq1K318A mutant proteins, after which their sensitivities to DNA-damaging agents were assessed. Multi-copy expression of wild-type Hrq1 protein did not affect the growth of yeast cells on control plates but rather suppressed the 4-NQO and cisplatin sensitivities of *hrq1Δ* cells (Fig. 1B). Overexpression of Hrq1K318A mutant protein did not reduce growth either, indicating that elevated levels of Hrq1 wild-type and Hrq1K318A mutant proteins were not harmful to yeast cells. However, overexpression of Hrq1K318A strongly inhibited growth of *hrq1Δ* strain in the presence of 4-NQO and cisplatin at much lower levels than those used in Fig. 1A and rendered cells as slightly sensitive to UV-irradiation (Fig. 1B). In contrast, overexpression of Hrq1K318A

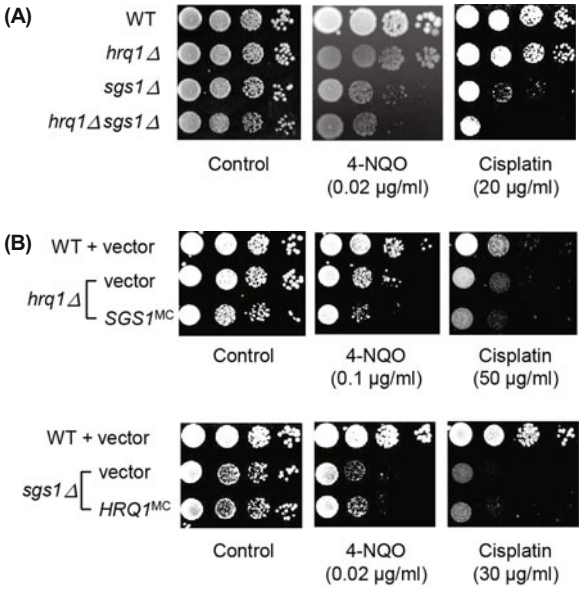

Fig. 2. Synergistic effects of *hrq1Δ* and *sgs1Δ* on sensitivities to 4-NQO and cisplatin. (A) Strains YPH499 (WT), YSH40 (*hrq1Δ*), YDH920 (*sgs1Δ*), and YSH45 (*hrq1Δ sgs1Δ*) were spotted in serial 10-fold dilutions onto SD media containing 4-NQO (0.02 μg/ml) or cisplatin (20 μg/ml), followed by incubation for 3 days at 30°C. (B) Complementation tests by multi-copy expression of *HRQ1* and *SGS1*. Strains YSH40 (*hrq1Δ*) and YSH45 (*sgs1Δ*) were transformed with multi-copy plasmids pRS325-*ADH1-SGS1* ($SGS1^{MC}$) and pRS325-*ADH1-HRQ1* ($HRQ1^{MC}$), respectively. Transformants were grown to saturation, and serial dilutions were spotted onto SD media containing the indicated amounts of 4-NQO and cisplatin. Vector alone controls (vector) indicate YPH499 (WT), YSH40 (*hrq1Δ*), and YSH45 (*sgs1Δ*) transformed with pRS325-*ADH1*.

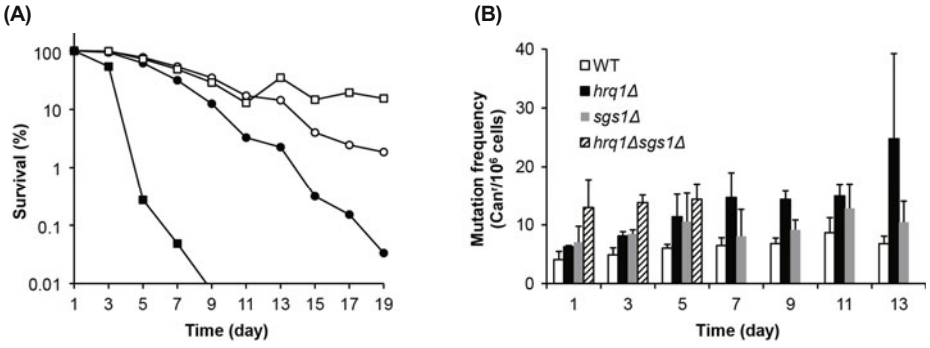

Fig. 3. Chronological survival and age-dependent mutation frequencies of *hrq1Δ* and *sgs1Δ*. Chronological survival (A) and age-dependent spontaneous mutation frequencies in *CAN1* gene (B) of YPH499 (wild-type, ○), YSH40 (*hrq1Δ*, ●), YDH920 (*sgs1Δ*, □), and YSH45 (*hrq1Δ sgs1Δ*, ■) were measured in SD media as described in 'Materials and Methods'. The averages and standard deviations from five independent experiments are shown.

protein did not alter the sensitivity to MMS or HU, implying that the increased sensitivities to 4-NQO and cisplatin are not due to general toxicity of protein overexpression. The dominant negative phenotype of *hrq1K318A* on 4-NQO or cisplatin sensitivity implies that Hrq1 is involved in a minor DNA damage repair pathway, and the elevated level of mutant Hrq1 protein impedes other major repair pathways by occupying the damaged sites, thereby hindering the access of other repair proteins. Hrq1 is not likely to have a major role in 4-NQO or cisplatin-induced DNA damage repair since the phenotype of the *hrq1Δ* mutant was not strong, and single-copy expression of *hrq1K318A* did not exhibit a dominant negative phenotype (compare Figs. 1A and 1B).

Multi-copy expression of Sgs1 does not complement the 4-NQO- and cisplatin-sensitive phenotype of *hrq1Δ* mutant

We next compared DNA-damaging agent sensitivities of the *hrq1Δ sgs1Δ* double mutant with those of respective single mutants. The *sgs1Δ* single mutant was much more sensitive to 4-NQO and cisplatin than *hrq1Δ* (Fig. 2A). The growth of *sgs1Δ* was strongly inhibited at much lower levels of 4-NQO (0.02 μg/ml) and cisplatin (20 μg/ml) (compare Figs. 1 and 2). This indicates that Sgs1 plays an important role in DNA damage repair induced by these chemicals. Although the *hrq1Δ* single mutation had no detectable effect at low levels of 4-NQO and cisplatin, the *hrq1Δ sgs1Δ* double mutant displayed an additive effect. The double mutant was synergistically more sensitive than either of the single mutants (Fig. 2A), suggesting that Hrq1 functions independently of Sgs1 in DNA repair pathways against 4-NQO- and cisplatin-induced DNA damage. However, the *hrq1Δ sgs1Δ* mutant did not display a synergistic growth defect upon MMS treatment (0.02%) or UV-irradiation (50 J/m^2) (data not shown), implying that Hrq1 is not involved in repair of MMS- or UV-induced DNA damage.

The data shown in Fig. 2A do not rule out the possibility that Hrq1 and Sgs1 have overlapping functions in repair pathways. To explore this possibility, we performed complementation tests using multi-copy plasmids expressing Hrq1 or Sgs1 proteins. We found that multi-copy expression of Sgs1 did not suppress the susceptibility of *hrq1Δ* cells to 4-NQO or cisplatin, and vice versa (Fig. 2B). Overexpression of Sgs1 was rather slightly inhibitory to cell growth. These results demonstrate that Hrq1 and Sgs1 cannot displace each other and have independent and different roles in DNA repair.

Hrq1 and Sgs1 function in parallel pathways for regulation of CLS and maintenance of genome stability

RTS is the best characterized of human RECQL4 diseases, and patients display many symptoms, including premature aging and susceptibility to cancer development (Bernstein *et al*., 2010). On the basis of sequence homology and genetic evidence (Barea *et al*., 2008; Groocock *et al*., 2012), Hrq1 is thought to be functional orthologue of RECQL4 in yeast. Thus we surmised that mutation of HRQ1 might also affect the life span of yeast cells. To explore this possibility, we compared CLS of wild-type strain with those of the *hrq1Δ* and *sgs1Δ* strains. While wild-type and *sgs1Δ* cells showed similar CLS, *hrq1Δ* cells displayed a consistently shortened CLS in multiple independent experiments (Fig. 3A). Unexpectedly, additional deletion of *SGS1* in *hrq1Δ* resulted in a dramatically shortened CLS, although *sgs1Δ* single mutation did not affect CLS. The viability of a 5-day-old *hrq1Δ sgs1Δ* double mutant was less than 1%, whereas more than 75% of wild-type cells of the same age were viable. This result indicates that both Hrq1 and Sgs1 have important roles in delaying chronological aging. However, we cannot exclude the possibility that shortened CLS is due to general sickness of the double mutant rather than chronological aging.

Mutations of RecQ helicases generally increase genomic instability (Seki *et al*., 2008; Chu and Hickson, 2009; Croteau *et al*., 2012). In the case of human RECQL4, cancer cells from RTS patients show increased chromosomal rearrangement (Bernstein *et al*., 2010). Mutations in *SGS1* increase the rate of accumulating gross chromosomal rearrangement (GCR) in yeast (Myung *et al*., 2001). Our results in Table 2 suggest that Hrq1 may also play a role in the maintenance of genome stability in that *hrq1Δ* and *sgs1Δ* mutations displayed a synergistic hyper-recombination phenotype. Thus, the shortened CLS of the *hrq1Δ sgs1Δ* double mutant may be a consequence of increased genome instability. To gain further insights into genome instability during chronological aging of *hrq1Δ* cells, we measured spontaneous mutation frequencies by monitoring Can^r mutations as described in Materials and Methods. The mutation frequency of *hrq1Δ* strain was about 1.5-fold higher than wild-type strain on day 1 of chronological aging, and it increased progressively with age to about 6~8-fold higher on day 13 (Fig. 3B). The *sgs1Δ* mutant also displayed higher mutation frequency than wild-type strain, but the age-dependent increase in mutation frequency was similar to that of wild-type. This discrepancy between the *hrq1Δ* and *sgs1Δ* mutants resulted in

Table 3. Spectrum of mutations observed in Can^r colonies from day 7

	Mutation type				Mutation type		
	Wild type	Nucleotide	Amino acid		*hrq1Δ*	Nucleotide	Amino acid
1	Transition	G→A (550)[a]	E→K (183)	1	Deletion	AD (969)	Frameshift
2	Transversion	G→C (361)	G→R (121)	2	Transition	C→T (899)	T→I (300)
3	Transition	G→A (1262)	G→D (421)	3	Transversion	A→T (1180)	I→F (393)
4	Transversion	G→C (1189)	G→R (397)	4	Transition	C→T (899)	T→I (300)
5	Transition Deletion	A→G (790) AΔ (794)	R→G (264) Frameshift	5	Transversion	A→C894	Q→H (298)
				6	Transition	T→C1213	S→P (405)
6	Transversion	T→A (146)	L→Stop (49)	7	Unidentified		
7	Deletion	GΔ (544)	Frameshift	8	Unidentified		
8	Transition	G→A (958)	A→T (320)	9	Transition	T→C1213	S→P (405)
9	Transversion	G→T (550)	E→Y (184)	10	Transition	T→C484	S→P (162)
10	Transition	G→A (389)	G→D (130)				

[a] The numbers in parentheses are positions of mutations from ATG or the first amino acid.

higher mutation frequency in *hrq1Δ* at the late stage of chronological aging. Consistent with the synergistic effects of *hrq1Δ* and *sgs1Δ* mutations on recombination frequencies (see Table 2), the spontaneous mutation frequency also synergistically increased in the *hrq1Δ sgs1Δ* double mutant (Fig. 3B), but we were unable to measure the mutation frequency after day 7 due to the extremely low chronological survival of the double mutant (Fig. 3A). Our results shown in Fig. 3 also demonstrate that Hrq1 and Sgs1 function in parallel pathways for the maintenance of genome stability.

To characterize the types of age-dependent mutations during chronological aging, we analyzed mutations in Can^r mutant colonies. For this purpose, we PCR-amplified and sequenced the *CAN1* genes in Can^r colonies from 7 day-old cultures. When *CAN1* ORFs were amplified in 50 Can^r colonies from *sgs1Δ*, only 42 colonies gave rise to the PCR products (data not shown). We were not able to obtain any PCR products in the remaining eight Can^r colonies even in the presence of various combinations of primers annealed to 5′- and 3′-flanking regions of *CAN1* ORF (data not shown). This is consistent with the previous observation that many Can^r mutants from *sgs1Δ* contain GCRs that prevent PCR-amplification of *CAN1* gene (Madia *et al.*, 2008). In contrast, intact *CAN1* genes were successfully PCR-amplified in all 50 Can^r colonies from *hrq1Δ* (data not shown), indicating that they were all point mutations rather than gross deletions. Therefore, Hrq1 is not likely to be involved in the suppression of GCRs during chronological aging. Sequence analyses of 10 randomly selected *CAN1* PCR products from *hrq1Δ* Can^r colonies revealed that eight mutants carried a single point mutation (Table 3). The remaining two mutants contained no mutations in the *CAN1* ORF. Since we analyzed only ORF regions, they may carry mutations in non-coding regions of *CAN1* gene. One Can^r mutant from *hrq1Δ* contained a single nucleotide deletion mutation, whereas the others contained base substitutions. Wild-type and *hrq1Δ* showed different patterns of base substitutions. In *hrq1Δ*, the majority of base substitutions were C↔T transitions, whereas most mutations in wild-type were G↔A transitions (Table 3). The importance of this discrepancy could not be elucidated at present.

Discussion

In this study, we have presented evidence that Hrq1 and Sgs1 function in parallel pathways in mitotic recombination and DNA repair. Firstly, *hrq1Δ* and *sgs1Δ* have synergistic effects on hyper-recombination and mutator phenotypes. Although the mutant phenotype of *hrq1Δ* alone was not strong, the *hrq1Δ sgs1Δ* double mutant clearly displayed elevated frequencies of recombination and spontaneous mutation (Table 2 and Fig. 3). Secondly, the sensitivity of *hrq1Δ* mutant to 4-NQO and cisplatin was not rescued by overexpression of Sgs1, although the *sgs1Δ* mutant was much more sensitive to these chemicals than the *hrq1Δ* mutant (Fig. 2). This result suggests that the defect of *hrq1Δ* is distinct from that of *sgs1Δ*. Thirdly, many age-dependent mutations in *sgs1Δ* arose from GCR, whereas we were only able to detect point mutations in *hrq1Δ* Can^r colonies (Table 3), implying that Hrq1 functions to suppress point mutations but not gross deletion mutations. Taken together, our results indicate that Hrq1 contributes to genome stability in a different way from Sgs1.

Sgs1 is a versatile and vigorous helicase that utilizes a broad range of DNA substrates, although it prefers Holliday junctions (Cejka and Kowalczykowski, 2010). The unwinding of various DNA intermediates generated during HR is the key function required for suppression of illegitimate recombination. Sgs1 has been proven to be a suitable enzyme for this activity (Cejka and Kowalczykowski, 2010). Our results that the frequency of mitotic recombination is elevated in *hrq1Δ* (Table 2) suggest that Hrq1 also plays a role in the suppression of illegitimate recombination. However, the substrates for Hrq1 are restricted to partial duplex DNA containing a long 3′-ssDNA tail (Kwon *et al.*, 2012). Hrq1 is absolutely dependent on the length of the 3′-tail, and no substrates without a long 3′-tail were unwound by Hrq1 in this study. This biochemical property suggests that Hrq1 is unlikely to unwind DNA intermediates of recombination lacking a 3′-tail. The purified *S. pombe* Hrq1 has been shown to readily unwind a bubble structure (Groocock *et al.*, 2012), whereas *S. cerevisiae* Hrq1 has not (Kwon *et al.*, 2012). Thus, we speculate that *S. cerevisiae* Hrq1 may require specific DNA structures for substrate targeting. A recent study showed that human RECQL4, which is most homologous to yeast

Hrq1, can displace telomeric D-loops (Ghosh *et al*., 2012). This observation hints that *S. cerevisiae* Hrq1 may unwind D-loops rather than bubble structures. Otherwise, Hrq1 may require specific interactions with proteins involved in HR for activation of its helicase activity.

RecQ helicases are involved in various DNA repair pathways, including base excision repair (BER), DSB repair, and inter-strand cross-link (ICL) repair (Singh *et al*., 2012). Although the involvement of RecQ helicases in NER is not clear yet, one finding has suggested a role for human RECQL4 in NER of UV- and 4-NQO-induced DNA damage (Fan and Luo, 2008). In this study, RECQL4 formed discrete nuclear foci in response to UV-irradiation and 4-NQO. In addition, RECQL4 directly interacted with XPA, a key protein involved in NER, and UV-irradiation induced nuclear colocalization of these two proteins. This is consistent with the UV-sensitive phenotype of human RTS cells lacking RECQL4 function. Hrq1 also appears to be implicated in NER. Epistasis analyses between *S. pombe* Hrq1 and ICL repair genes in response to cisplatin-induced DNA damage suggested that *S. pombe* Hrq1 functions within the NER pathway involving Rad16 and Rhp14 (Groocock *et al*., 2012). Considering that UV- and 4-NQO-induced DNA damage is primarily repaired by NER (Jones *et al*., 1989; Ide *et al*., 2001), 4-NQO sensitivity of *S. cerevisiae hrq1Δ* (Fig. 1A) also implies the involvement of Hrq1 in NER. Although the *hrq1Δ* mutant did not display a UV-sensitive phenotype, overexpression of Hrq1K318A mutant protein rendered yeast cells to be slightly sensitive to UV-irradiation (Fig. 1B), suggesting that the repair mechanism of UV-induced DNA damage is partially impaired by *hrq1* mutation. In conclusion, we speculate that Hrq1 probably plays an auxiliary role in NER. To test this hypothesis, further studies are required such as epistasis analysis and identification of Hrq1-interacting proteins. Hrq1 has advantages over human RECQL4 for biochemical and genetic studies of these enzymes. Therefore, studies on Hrq1 will provide important clues for understanding the *in vivo* functions of RECQL4 and the genetic diseases linked to RECQL4 mutations.

Acknowledgements

This work was supported by National Research Foundation of Korea (2012R1A1A2009053) and Inha University.

References

Ashton, T.M. and Hickson, I.D. 2010. Yeast as a model system to study RecQ helicase function. *DNA Repair (Amst)* **9**, 303–314.

Barea, F., Tessaro, S., and Bonatto, D. 2008. *In silico* analyses of a new group of fungal and plant RecQ4-homologous proteins. *Comput. Biol. Chem.* **32**, 349–358.

Baudin, A., Ozier-Kalogeropoulos, O., Denouel, A., Lacroute, F., and Cullin, C. 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* **21**, 3329–3330.

Bernstein, K.A., Gangloff, S., and Rothstein, R. 2010. The RecQ DNA helicases in DNA repair. *Annu. Rev. Genet.* **44**, 393–417.

Bohr, V.A. 2008. Rising from the RecQ-age: the role of human RecQ helicases in genome maintenance. *Trends Biochem. Sci.* **33**, 609–620.

Cejka, P. and Kowalczykowski, S.C. 2010. The full-length *Saccharomyces cerevisiae* Sgs1 protein is a vigorous DNA helicase that preferentially unwinds holliday junctions. *J. Biol. Chem.* **285**, 8290–8301.

Chu, W.K. and Hickson, I.D. 2009. RecQ helicases: multifunctional genome caretakers. *Nat. Rev. Cancer* **9**, 644–654.

Chris, K., Michaelis, S., and Mitchell, A. 1994. Methods in yeast genetics. pp. 207–217. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, N.Y., USA.

Cobb, J.A., Schleker, T., Rojas, V., Bjergbaek, L., Tercero, J.A., and Gasser, S.M. 2005. Replisome instability, fork collapse, and gross chromosomal rearrangements arise synergistically from Mec1 kinase and RecQ helicase mutations. *Genes Dev.* **19**, 3055–3069.

Croteau, D.L., Singh, D.K., Hoh Ferrarelli, L., Lu, H., and Bohr, V.A. 2012. RECQL4 in genomic instability and aging. *Trends Genet.* **28**, 624–631.

Davies, S.L., North, P.S., and Hickson, I.D. 2007. Role for BLM in replication-fork restart and suppression of origin firing after replicative stress. *Nat. Struct. Mol. Biol.* **14**, 677–679.

Fan, W. and Luo, J. 2008. RecQ4 facilitates UV light-induced DNA damage repair through interaction with nucleotide excision repair factor xeroderma pigmentosum group A (XPA). *J. Biol. Chem.* **283**, 29037–29044.

Ghosh, A.K., Rossi, M.L., Singh, D.K., Dunn, C., Ramamoorthy, M., Croteau, D.L., Liu, Y., and Bohr, V.A. 2012. RECQL4, the protein mutated in Rothmund-Thomson syndrome, functions in telomere maintenance. *J. Biol. Chem.* **287**, 196–209.

Groocock, L.M., Prudden, J., Perry, J.J., and Boddy, M.N. 2012. The RecQ4 orthologue Hrq1 is critical for DNA interstrand cross-link repair and genome stability in fission yeast. *Mol. Cell. Biol.* **32**, 276–287.

Ide, F., Oda, H., Nakatsuru, Y., Kusama, K., Sakashita, H., Tanaka, K., and Ishikawa, T. 2001. Xeroderma pigmentosum group A gene action as a protection factor against 4-nitroquinoline 1-oxide-induced tongue carcinogenesis. *Carcinogenesis* **22**, 567–572.

Jones, C.J., Edwards, S.M., and Waters, R. 1989. The repair of identified large DNA adducts induced by 4-nitroquinoline-1-oxide in normal or xeroderma pigmentosum group A human fibroblasts, and the role of DNA polymerases alpha or delta. *Carcinogenesis* **10**, 1197–1201.

Kadyk, L.C. and Hartwell, L.H. 1992. Sister chromatids are preferred over homologs as substrates for recombinational repair in *Saccharomyces cerevisiae*. *Genetics* **132**, 387–402.

Kwon, S.H., Choi, D.H., Lee, R., and Bae, S.H. 2012. *Saccharomyces cerevisiae* Hrq1 requires a long 3′-tailed DNA substrate for helicase activity. *Biochem. Biophys. Res. Commun.* **427**, 623–628.

Madia, F., Gattazzo, C., Wei, M., Fabrizio, P., Burhans, W.C., Weinberger, M., Galbani, A., Smith, J.R., Nguyen, C., Huey, S., and *et al.* 2008. Longevity mutation in SCH9 prevents recombination errors and premature genomic instability in a Werner/Bloom model system. *J. Cell. Biol.* **180**, 67–81.

Mandell, J.G., Goodrich, K.J., Bahler, J., and Cech, T.R. 2005. Expression of a RecQ helicase homolog affects progression through crisis in fission yeast lacking telomerase. *J. Biol. Chem.* **280**, 5249–5257.

Mimitou, E.P. and Symington, L.S. 2008. Sae2, Exo1 and Sgs1 collaborate in DNA double-strand break processing. *Nature* **455**, 770–774.

Monnat, R.J. Jr. 2010. Human RECQ helicases: roles in DNA metabolism, mutagenesis and cancer biology. *Semin. Cancer Biol.* **20**, 329–339.

Myung, K., Datta, A., Chen, C., and Kolodner, R.D. 2001. *SGS1*, the *Saccharomyces cerevisiae* homologue of BLM and WRN, suppresses genome instability and homeologous recombination. *Nat. Genet.* **27**, 113–116.

Onoda, F., Seki, M., Miyajima, A., and Enomoto, T. 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., Seki, M., Wang, W., and Enomoto, T. 2004a. The hyper unequal sister chromatid recombination in an sgs1 mutant of budding yeast requires MSH2. *DNA Repair (Amst)* **3**, 1355–1362.

Onoda, F., Takeda, M., Seki, M., Maeda, D., Tajima, J., Ui, A., Yagi, H., and Enomoto, T. 2004b. SMC6 is required for MMS-induced interchromosomal and sister chromatid recombinations in *Saccharomyces cerevisiae*. *DNA Repair (Amst)* **3**, 429–439.

Resnick, M.A., Sugino, A., Nitiss, J., and Chow, T. 1984. DNA polymerases, deoxyribonucleases, and recombination during meiosis in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **4**, 2811–2817.

Seki, M., Otsuki, M., Ishii, Y., Tada, S., and Enomoto, T. 2008. RecQ family helicases in genome stability: lessons from gene disruption studies in DT40 cells. *Cell Cycle* **7**, 2472–2478.

Sikorski, R.S. and Hieter, P. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**, 19–27.

Singh, D.K., Ghosh, A.K., Croteau, D.L., and Bohr, V.A. 2012. RecQ helicases in DNA double strand break repair and telomere maintenance. *Mutat. Res.* **736**, 15–24.

Watt, P.M., Hickson, I.D., Borts, R.H., and Louis, E.J. 1996. *SGS1*, a homologue of the Bloom's and Werner's syndrome genes, is required for maintenance of genome stability in *Saccharomyces cerevisiae*. *Genetics* **144**, 935–945.

Wu, L. 2007. Role of the BLM helicase in replication fork management. *DNA Repair (Amst)* **6**, 936–944.

Zhu, Z., Chung, W.H., Shim, E.Y., Lee, S.E., and Ira, G. 2008. Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* **134**, 981–994.