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Hrq1 Facilitates Nucleotide Excision Repair of DNA Damage Induced by 4-Nitroquinoline-1-Oxide and Cisplatin in *Saccharomyces cerevisiae*

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Hrq1 helicase is a novel member of the RecQ family. Among the five human RecQ helicases, Hrq1 is most homologous to RECQL4 and is conserved in fungal genomes. Recent genetic and biochemical studies have shown that it is a functional gene, involved in the maintenance of genome stability. To better define the roles of Hrq1 in yeast cells, we investigated genetic interactions between *HRQ1* and several DNA repair genes. Based on DNA damage sensitivities induced by 4-nitroquinoline-1-oxide (4-NQO) or cisplatin, *RAD4* was found to be epistatic to *HRQ1*. On the other hand, mutant strains defective in either homologous recombination (HR) or post-replication repair (PRR) became more sensitive by additional deletion of *HRQ1*, indicating that *HRQ1* functions in the *RAD4*-dependent nucleotide excision repair (NER) pathway independent of HR or PRR. In support of this, yeast two-hybrid analysis showed that Hrq1 interacted with Rad4, which was enhanced by DNA damage. Overexpression of Hrq1K318A helicase-deficient protein rendered mutant cells more sensitive to 4-NQO and cisplatin, suggesting that helicase activity is required for the proper function of Hrq1 in NER.

Keywords: Hrq1, RecQ helicase, DNA helicase, RECQL4 orthologue, nucleotide excision repair, Rad4

Introduction

Nucleotide excision repair (NER) is a highly conserved and versatile DNA repair mechanism for DNA damage that creates helix distortions, such as UV photoproducts and bulky adducts (Prakash and Prakash, 2000). NER is divided into two pathways: global genome repair (GGR) and transcription-coupled repair (TCR). These two pathways are operated by different subsets of NER proteins during the initial steps of DNA damage recognition but share common NER factors in the downstream steps, which include lesion verification, helix opening, and dual incision of the damaged strand (Prakash and Prakash, 2000; Compe and Egly, 2012).

In *Saccharomyces cerevisiae*, only GGR has been shown to be dependent on Rad7, Rad16, and Elc1 (Verhage *et al.*, 1994; Lejeune *et al.*, 2009). The Rad7-Rad16 complex initially binds to a damage site in an ATP-dependent manner and then serves as a nucleation site for the recruitment of other NER factors (Prakash and Prakash, 2000). Although the precise role of Elc1 is not yet clear, it seems to play an important role in GGR that is not subsidiary to that of Rad7 or Rad16 (Lejeune *et al.*, 2009). TCR is triggered by stalled RNA polymerase II at DNA lesions in the transcribed strand (Compe and Egly, 2012). Whereas there seems to be only one TCR pathway in *Escherichia coli*, TCR in eukaryotes diverges into two subpathways mediated by Rad26/CSB (yeast/human) and Rpb9 (Li and Smerdon, 2004).

After initial recognition of lesions, Rad4/XPC (yeast/human) binds to the DNA damage site and promotes repair processes (Prakash and Prakash, 2000). The Rad4/XPC-damaged DNA complex subsequently recruits the transcription initiation factor TFIIH (Riedl *et al.*, 2003; Lafrance-Vanasse *et al.*, 2013), which verifies and unwinds the damaged DNA region using its DNA helicase subunits (Oksenych and Coin, 2010; Compe and Egly, 2012). Rad14/XPA (yeast/human) is another DNA damage-binding protein and arrives together with RPA at the Rad4/XPC-TFIIH-damaged DNA complex (Mardiros *et al.*, 2011; Compe and Egly, 2012). RPA and Rad14/XPA are believed to function in the stabilization of unwound damaged DNA as well as recruitment of the NER-specific endonucleases Rad1-Rad10/XPF-ERCC1 (yeast/human) and Rad2/XPG (yeast/human), which are responsible for 5′ and 3′ incisions of the DNA damage site, respectively (Guzder *et al.*, 2006; Tsodikov *et al.*, 2007; Mardiros *et al.*, 2011).

Homologous recombination (HR) is crucial to the repair of DNA double-strand breaks, damaged replication forks, and DNA interstrand crosslinks in mitotic cells (Pâques and Haber, 1999). Rad51 is the eukaryotic RecA orthologue that catalyzes homologous pairing and DNA strand invasion during the early steps of HR. The activity of Rad51 is facilitated and regulated by the recombination mediator proteins including Rad52, Rad54, Rad55, and rad57 (Sugawara *et al.*, 2003; Maher *et al.*, 2011). Rad52 is the most-studied mediator protein that promotes Rad51 presynaptic filament assembly and RPA displacement from single-stranded DNA (Lok and Powell, 2012). Considering the *RAD52* epistasis group includes many HR genes, it is not surprising that *RAD52* is involved in all known HR pathways.

Postreplication repair (PRR) pathways are lesion bypass

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processes activated by stalled DNA replication forks (Lee and Myung, 2008; Zhang *et al*., 2011). There are two known pathways in PRR. The error-prone pathway known as translesion synthesis (TLS) uses specialized DNA polymerases for bypassing DNA lesions. In *S. cerevisiae*, TLS is primarily catalyzed by DNA polymerases η (Rad30) or ζ (Rev3-Rev7). The error-free lesion bypass pathway is dependent on Rad5, a multi-functional protein possessing both ubiqutin ligase and DNA helicase activities (Blastyák *et al*., 2007; Zhang *et al*., 2011). This pathway is activated by polyubiqutination of PCNA catalyzed by E2 enzyme (Mms2-Ubc13) and E3 ligase Rad5. Although the exact mechanism remains unclear, error-free replication through DNA lesion occurs by template switching or replication fork regression. It was proposed that Rad5 helicase activity is required for fork regression *in vitro* (Blastyák *et al*., 2007).

The RecQ helicase family is highly conserved in all living organisms and plays multiple roles in the maintenance of genome stability. These proteins are involved in the various steps of HR as well as DNA repair pathways (Chu and Hickson, 2009; Bernstein *et al*., 2010). There are five RecQ helicases in humans, designated RECQL1, BLM, WRN, RECQL4, and RECQL5. BLM and WRN are linked to the 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, 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).

Sgs1 and Rqh1, similar to human BLM, have long been considered as the only RecQ family members in *S. cerevisiae* and *Schizosaccharomyces pombe*, respectively (Ashton and Hickson, 2010; Bernstein *et al*., 2010). However, bioinformatics analyses have recently predicted the presence of another highly conserved RecQ-homologous protein, Hrq1, in the fungal and plant genomes, which is most similar to human RECQL4 (Barea *et al*., 2008). This prediction has been supported by recent studies in *S. pombe* and *S. cerevisiae*. Specifically, genetic studies in *S. pombe* have shown that Hrq1-deficient mutant cells suffer spontaneous genome instability and exhibit hyper-recombination along with mutator phenotypes (Groocock *et al*., 2012). It was proposed based on genetic analyses that *S. pombe* Hrq1 is involved in NER pathway, playing a role in repair of cisplatin-induced DNA damage.

More 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 as well as DNA strand-annealing activities, similar to other RecQ helicases. We also characterized the phenotypes of *hrq1Δ* mutation (Choi *et al*., 2013). In detail, it was shown that *hrq1Δ* mutants display increased mitotic recombination and spontaneous mutation frequencies, and their growths were significantly inhibited by treatment with 4-nitroquinoline-1-oxide (4-NQO) or cisplatin, which was not complemented by Sgs1 overexpression. Further, the *hrq1Δ sgs1Δ* double mutant exhibits a shortened chronological lifespan compared to respective single mutants. Based on these observations, Hrq1 is likely involved in recombination and repair pathways independent of Sgs1 helicase.

To further characterize the roles of Hrq1 in recombination and repair pathways, we performed epistasis analyses between *HRQ1* and various repair and recombination genes. Our genetic results indicate that *RAD4* is epistatic to *HRQ1*, suggesting *HRQ1* functions with *RAD4* in a common NER pathway. In support of this, yeast two-hybrid analysis shows

Table 1. **Yeast strains used in this study**

Strain	Genotype	References
YPH499	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	Sikorski and Hieter (1989)
YSH40	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hrq1Δ::HIS3*	This study
YSH61	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad4Δ::TRP1*	This study
YSH93	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad4Δ::TRP1 hrq1Δ::HIS3*	This study
YSH57	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad10Δ::TRP1*	This study
YSH80	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad10Δ::TRP1 hrq1Δ::HIS3*	This study
YSH67	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad51Δ::LEU2*	This study
YSH92	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad51Δ::LEU2 hrq1Δ::HIS3*	This study
YDH170	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad52Δ::URA3*	This study
YDH171	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad52Δ::URA3 hrq1Δ::HIS3*	This study
YDH110	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad5Δ::TRP1*	This study
YDH111	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad5Δ::TRP1 hrq1Δ::URA3*	This study
YSH60	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rev3Δ::TRP1*	This study
YSH92	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rev3Δ::TRP1 hrq1Δ::HIS3*	This study
YDH164	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad26Δ::TRP1*	This study
YDH165	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad26Δ::TRP1 hrq1Δ::HIS3*	This study
YDH181	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rad7Δ::TRP1*	This study
YDH182	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hrq1Δ::HIS3 rad7Δ::TRP1*	This study
YDH189	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb9Δ::URA3*	This study
YDH190	*MATa ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hrq1Δ::HIS3 rpb9Δ::URA3*	This study
PJ69-4A	*MATa trp1-901 leu2-3,112 ura3-52 his3-200 gal4Δ gal80Δ LYS2::GAL1-HIS3 GAL2-ADE2 met2::GAL7-lacZ*	James *et al.* (1996)

that Hrq1 interacts with Rad4, which is enhanced by DNA damage.

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 complete (SC) media, were prepared as described (Chris *et al.*, 1994). Gene disruptions were conducted by one-step gene displacement methods as previously described (Sikorski and Hieter, 1989; Baudin *et al.*, 1993). To disrupt each gene, selective markers were PCR-amplified using primers harboring 50 base-pairs homologous to the 5′ or 3′ flanking region of each open reading frame (ORF) and then transformed into yeast cells to replace the target gene. Yeast colonies grown in selective media were analyzed by PCR for correct gene disruption. The multi-copy plasmid pRS325-*ADH1-hrq1K318A* (Choi *et al.*, 2013) was used to overexpress the helicase-deficient Hrq1-K318A mutant protein in yeast cells.

Measurement of sensitivity to chemical agents

Yeast cells were grown to stationary phase in liquid SC media, diluted 1:1000, and then grown to mid-log phase until all strains reached a similar OD_{600}. For exposure to DNA-damaging agent, 10-fold serial dilutions of the cell culture from 10^7 cells/ml were spotted onto SC plates containing either 4-NQO (0.5–100 ng/ml) or cisplatin (20–50 μg/ml). The plates were incubated for 3–4 days at 30°C.

Yeast two-hybrid analysis

The ORFs of *HRQ1* and *RAD4* genes were amplified by PCR and cloned into *Bam*HI-*Pst*I sites of plasmid pGBD-C1 and *Eco*RI-*Pst*I sites of pGAD-C1 (James *et al.*, 1996), respectively, to generate plasmids pGBD-*HRQ1* and pGAD-*RAD4*. The resulting plasmids express *GAL4* DNA-binding domain (BD)-Hrq1 fusion and *GAL4* activation domain (AD)-Rad4 fusion proteins, respectively. For positive control experiments, the ORFs of *RNR2* and *RNR4* genes were PCR-amplified and cloned into *Bam*HI-*Pst*I site of pGAD-C1 and pGBD-C1, respectively. The plasmids were then transformed into *S. cerevisiae* strain PJ69-4A (James *et al.*, 1996) for two-hybrid analysis. PJ69-4A contains the *HIS3* reporter gene under control of the *GAL4* promoter, providing histidine prototropy based on interaction between bait and prey proteins. To detect two-hybrid interactions, yeast cells were grown in liquid SC media, followed by spotting onto SC plates lacking histidine. The plates were then incubated at 30°C for 3–5 days. In order to examine the effect of DNA damage on two-hybrid interactions, yeast cells were spotted onto SC plates containing either 4-NQO (10 ng/ml) or cisplatin (20 μg/ml).

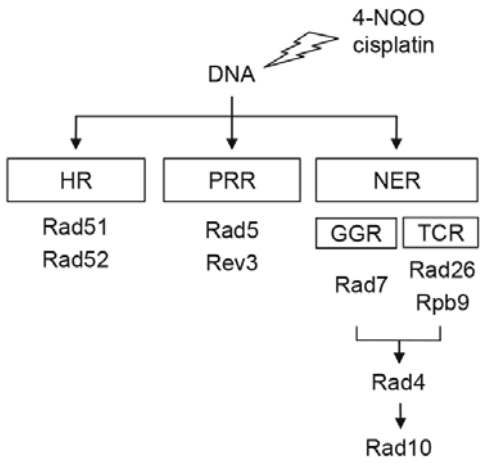

Fig. 1. Schematic representation of the simplest genetic relationship between the repair genes examined in this study. The lightning denotes DNA damage caused by 4-NQO or cisplatin. The repair pathways are indicated in the rectangles.

Results

HRQ1 functions in *RAD4*-dependent pathway

S. cerevisiae hrq1Δ mutants show mild sensitivity to DNA damages caused by 4-NQO or cisplatin, suggesting that Hrq1 may play an auxiliary role in DNA repair pathways (Choi *et al.*, 2013). Multiple pathways are involved in the repair of DNA lesions induced by 4-NQO or cisplatin, including HR, PRR, and NER (Ide *et al.*, 2001; McVey, 2010; Williams *et al.*, 2010). To investigate the precise role of Hrq1 in DNA repair, we examined genetic relationships between *HRQ1* and the genes playing crucial roles in these DNA repair pathways (Fig. 1). For this purpose, we constructed double mutant strains bearing *hrq1Δ* as well as deletion of each repair gene (Table 1), followed by examination of their sensitivities to DNA damaging agents.

Disruption of HR (*rad51Δ* and *rad52Δ*), PRR (*rad5Δ* and *rev3Δ*), or NER (*rad4Δ* and *rad10Δ*) rendered mutant cells hypersensitive to 4-NQO and cisplatin (Fig. 2). Growth of single mutant strains was strongly inhibited at low levels of 4-NQO (0.5–20 ng/ml) or cisplatin (5–30 μg/ml). In contrast, *hrq1Δ* mutant exhibited only moderate sensitivities even in the presence of much higher levels of 4-NQO (100 ng/ml) or cisplatin (40 μg/ml). Although *hrq1Δ* mutant did not show any growth defects at low levels of 4-NQO or cisplatin (data not shown), the double mutant strains defective in HR or PRR (*hrq1Δ rad51Δ*, *hrq1Δ rad52Δ*, *hrq1Δ rad5Δ*, and *hrq1Δ rev3Δ*) were additively more sensitive to both 4-NQO and cisplatin compared to respective single mutants (Fig. 2). Thus, *HRQ1* is most likely to function in parallel to HR and PRR. In contrast, the growth defects of the *rad4Δ* and *rad10Δ* single mutants were not exacerbated by additional deletion of *HRQ1*. The 4-NQO and cisplatin sensitivities of the *rad4Δ hrq1Δ* and *rad10Δ hrq1Δ* double mutants were equivalent to those of *rad4Δ* and *rad10Δ*, respectively (Fig. 2). These results suggest that *HRQ1* acts in the same pathway as *RAD4* and *RAD10*. Since *RAD4* and *RAD10* are key players in the common NER pathway, we surmise that *HRQ1* functions in NER.

In *S. cerevisiae*, *RAD7* is specifically required for GGR, whereas *RAD26* and *RPB9* are specific for TCR (Verhage *et*

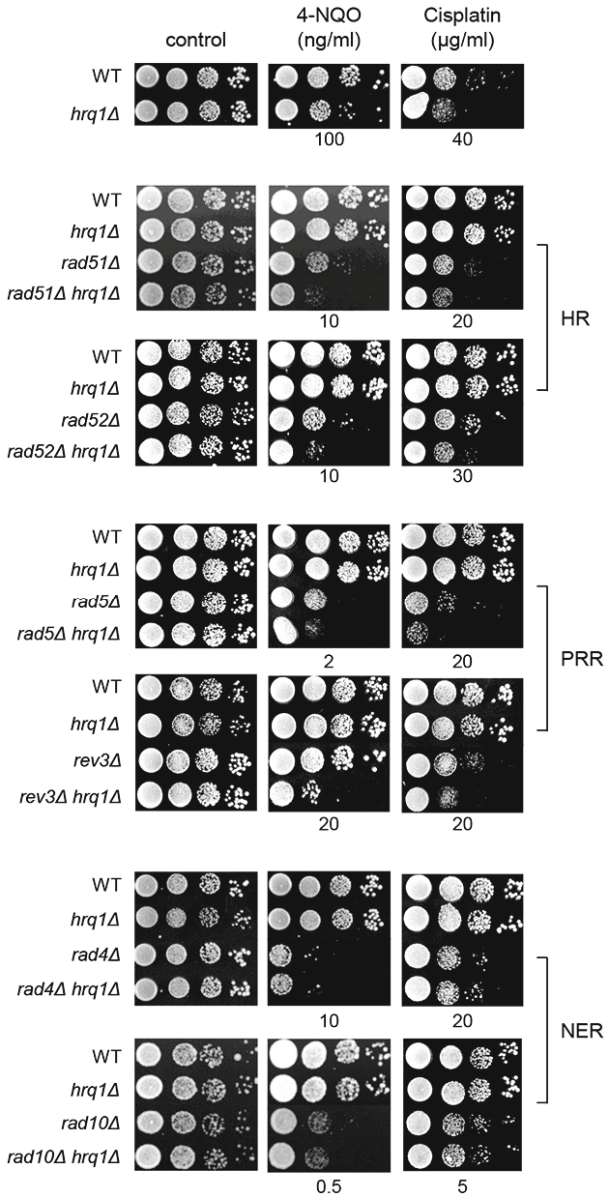

Fig. 2. Genetic interactions between *HRQ1* and various repair genes. Wild-type strain YPH499 (WT) and mutant strains of the indicated genotypes were spotted in serial 10-fold dilutions onto SC media containing the indicated amount of 4-NQO (0.5–100 ng/ml) or cisplatin (5–40 μg/ml), followed by incubation for 3 days at 30°C. The defective repair pathways caused by each mutation are denoted at the right of the figure.

al., 1994; Li and Smerdon, 2004). To determine whether or not *HRQ1* plays a role in either of these pathways, we introduced *hrq1Δ* mutation into *rad7Δ*, *rpb9Δ*, or *rad26Δ* mutant cells and then assessed sensitivities of the resulting double mutants to 4-NQO and ciplatin. Unlike the epistasis observed between *hrq1Δ* and *rad4Δ*, all double mutants were synergistically more sensitive to both 4-NQO and cisplatin compared to respective single mutants (Fig. 3). Thus, Hrq1 may facilitate a common step in GGR and TCR. Based on the results shown in Figs. 2 and 3, we surmise that *HRQ1* functions with *RAD4* but independent of HR and PRR.

Dominant negative effect of *hrq1K318A* is dependent on *RAD4*, but not *RAD10*

The epistatic relationships between *HRQ1* and NER genes (*RAD4* and *RAD10*) shown in Fig. 2 suggest that *HRQ1* functions in NER pathway. However, the mild phenotype of *hrq1Δ* single mutation makes any further genetic analysis difficult. To overcome this problem, we utilized the dominant negative effect of *hrq1K318A* helicase-deficient mutation. Previously, we observed that overexpression of Hrq1K318A mutant protein strongly inhibits growth of *hrq1Δ* strains in the presence of 4-NQO and cisplatin (Choi *et al.*, 2013). This prompted us to perform epistasis analyses under the condition of *hrq1K318A* overexpression. For this purpose, we transformed each mutant cell with either empty vector or a multi-copy plasmid expressing Hrq1K318A protein and then compared their susceptibilities to 4-NQO and cisplatin. Overexpression of Hrq1K318A did not affect the growth of yeast cells on control plates, indicating that overexpression of Hrq1K318A was not harmful to yeast cells under normal conditions (Fig. 4). However, all mutant strains, except for *rad4Δ hrq1Δ*, exhibited enhanced sensitivities to 4-NQO and cisplatin upon Hrq1K318A overexpression, suggesting that up-regulation of Hrq1K318A dominantly hinders the repair pathways for 4-NQO or cisplatin-induced DNA damage. In contrast, the deleterious effect of Hrq1K318A was not observed in *rad4Δ hrq1Δ* strain (Fig. 4), indicating that the dominant negative effect of Hrq1K318A is dependent on the presence of Rad4 protein. Consistent with the epistatic relationship between *HRQ1* and *RAD4*, these results suggest that *HRQ1* functions in *RAD4*-dependent pathway. The susceptibility of *rad10Δ hrq1Δ* double mutant was indistingui-

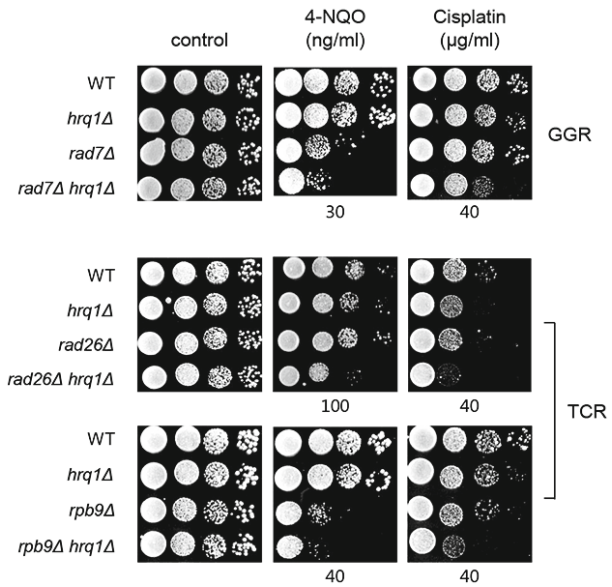

Fig. 3. Genetic interactions between *HRQ1* and the repair genes specific for GGR or TCR. Strains of the indicated genotypes were spotted in serial 10-fold dilutions onto SC media containing the indicated amount of 4-NQO (30–100 ng/ml) or cisplatin (40 μg/ml), followed by incubation for 3 days at 30°C. The defective repair pathways caused by each mutation are denoted at the right of the figure.

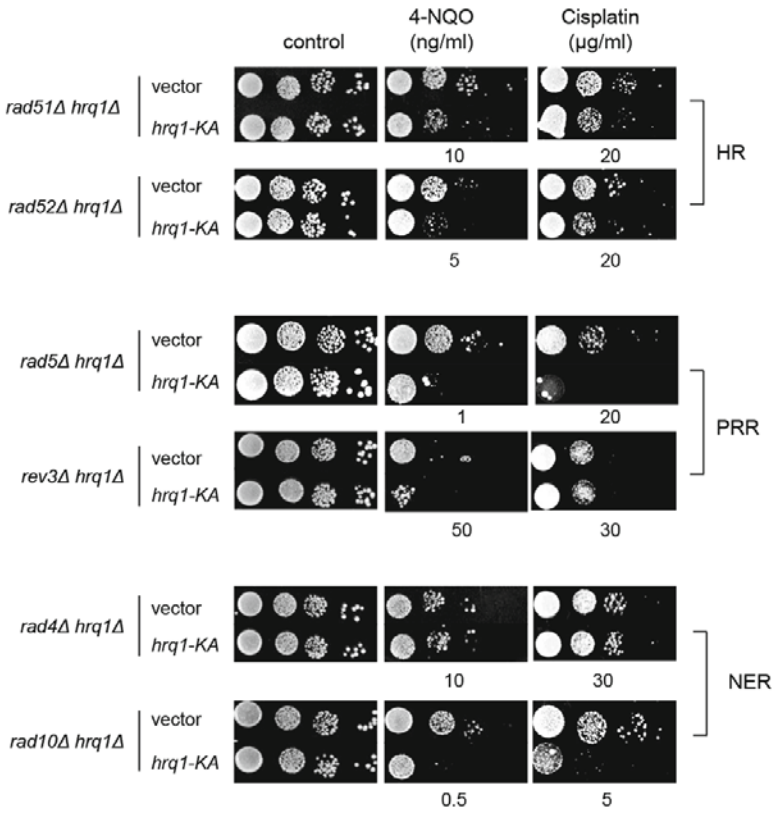

Fig. 4. Dominant negative effects of *hrq1K318A* mutation on DNA damage sensitivity. Strains of the indicated genotype were transformed with the multi-copy plasmid pRS325-*ADH1* (vector) or pRS325-*ADH1-hrq1K318A* (*hrq1-KA*). After transformants were grown to saturation, serial 10-fold dilutions were spotted onto SC media containing the indicated amount of 4-NQO (0.5–50 ng/ml) or cisplatin (5–30 μg/ml), followed by incubation for 3 days at 30°C. The defective repair pathways caused by each mutation are denoted at the right of the figure.

shable from that of *rad10Δ* single mutant (Fig. 2). However, multi-copy expression of Hrq1K318A rendered *rad10Δ hrq1Δ* cells more sensitive to 4-NQO and cisplatin (Fig. 4). This discrepancy may be due to the significant difference in the sensitivities to DNA damaging agents. As shown in Fig. 2, growth of *rad10Δ* strain was strongly inhibited at much lower concentrations of 4-NQO (0.5 ng/ml) or cisplatin (5 μg/ml) than those used for *hrq1Δ* (100 ng/ml and 20 μg/ml, respectively). Thus, we speculate that the phenotype of *hrq1Δ* is too weak for *rad10Δ hrq1Δ* to exhibit synergistic effects at low concentrations of DNA damaging agents. Based on the dominant negative effect of Hrq1K318A overexpression on *rad10Δ hrq1Δ*, *RAD10* is not likely to be epistatic to *HRQ1*.

Yeast two-hybrid interaction between Hrq1 and Rad4

Based on the epistasis relationship between *HRQ1* and *RAD4*, we examined whether or not Hrq1 protein directly interacts with Rad4. For this purpose, we constructed a yeast strain

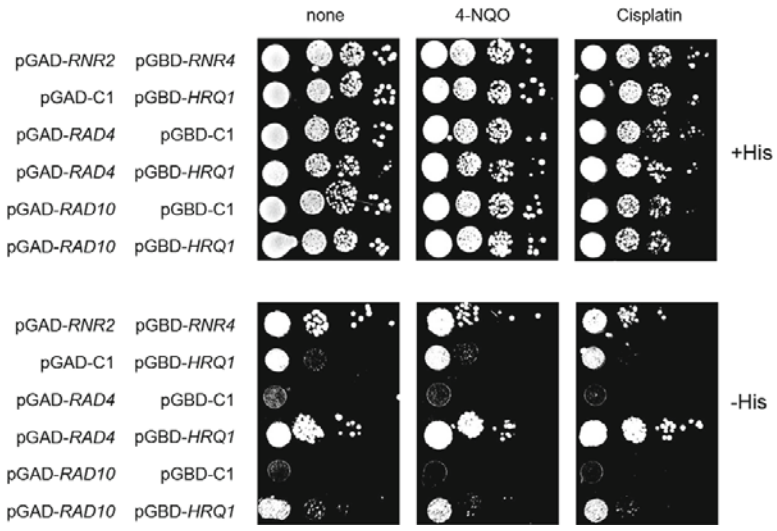

Fig. 5. Yeast two-hybrid interactions between Hrq1 and Rad14. The plasmids transformed into PJ69-4A are indicated at the left of the figure. After transformants were grown to saturation, serial 10-fold dilutions were spotted onto SC media plus histidine (+His) or minus histidine (-His), followed by incubation for 3–5 days at 30°C. To induce DNA damage, 4-NQO (10 ng/ml) or cisplatin (20 μg/ml) was added to the SC plates.

containing a tandem affinity purification (TAP) tag and hemagglutinin (HA) tag fused to the C-terminal regions of chromosomal *HRQ1* and *RAD4* genes, respectively. However, we failed to detect any physical interaction between Hrq1-TAP and Rad4-HA in the pull-down experiments (data not shown). Next, we conducted yeast two-hybrid analyses using BD-Hrq1 (pGBD-*HRQ1*), AD-Rad4 (pGAD-*RAD4*), and AD-Rad10 (pGAD-*RAD10*). pGAD-*RAD4* or pGAD-*RAD10* in combination with empty vector (pGBD-C1) hardly activate transcription of *HIS3*, whereas co-transformation of pGBD-*HRQ1* and pGAD-C1 resulted in basal level expression (Fig. 5). When pGBD-*HRQ1* and pGAD-*RAD4* were co-transformed, higher *HIS3* expression was observed compared to basal level expression induced by pGBD-*HRQ1* alone, indicating positive two-hybrid interaction between Hrq1 and Rad4. In contrast, growth of cells containing pGBD-*HRQ1* and pGAD-*RAD10* was almost similar to the background growth produced by pGBD-*HRQ1* alone. Thus, this result suggests that Hrq1 interacts with Rad4 but not with Rad10. Interestingly, the two-hybrid interaction between Hrq1 and Rad4 was enhanced by DNA damage in comparison with the positive control (pGAD-*RNR2* and pGBD-*RNR4*). In the presence of DNA damaging agents, reporter gene expression was somewhat reduced in the positive control but slightly elevated in yeast cells containing pGBD-*HRQ1* and pGAD-*RAD4* (Fig. 5). We suppose that activation of NER by DNA damage may enhance the protein interaction between Hrq1 and Rad4.

Discussion

In this study, we show that *HRQ1* functions with *RAD4* in NER. Epistasis analyses and dominant negative effect of *hrq1K318A* mutation revealed that only *RAD4* was epistatic to *HRQ1* among the repair genes examined in this study. In support of this, Hrq1 interacts with Rad4 in yeast two-hybrid analysis. However, Rad4 was not co-precipitated with Hrq1 in the pull-down experiment. As mentioned above, we used C-terminal epitope tags (Hrq1-TAP and Rad4-HA) in pull-down assay whereas N-terminal fusions in yeast two-hybrid assay (BD-Hrq1 and AD-Rad4). We conjecture that Rad4-HA was not co-precipitated with Hrq1-TAP possibly because the C-terminal tags sterically hindered the protein-protein interaction. If the C-terminal regions of Hrq1 and Rad4 are indeed important for their interaction, then obviously C-terminal tagging would cause disruption.

The two-hybrid interaction does not necessarily reflect direct interaction between Hrq1 and Rad4. We cannot rule out the possibility that other NER factor(s) mediates Hrq1-Rad4 interaction. Rad14 (human XPA) is one of such candidates. In human cells, it has been demonstrated that RECQL4 facilitates repair of UV-induced DNA damage through physical interaction with XPA (Fan and Luo, 2008). Consistent with this, *S. pombe* Hrq1 was co-immunoprecipitated with Rhp14 (human XPA, yeast Rad14) (Groocock *et al.*, 2012). However, since these studies did not examine direct interaction between Hrq1 and XPA/Rhp14, it is still possible that RECQL4/Hrq1 indirectly interacts with XPA/Rhp14. Considering that XPA associates with the N-terminal moiety of XPC (yeast Rad4) on the damaged site (Compe and Egly, 2012), *S. cerevisiae* Hrq1 may directly interact with either Rad14 or Rad4 or possibly both.

Recognition of DNA lesions by Rad4 is essential for both GGR and TCR in *S. cerevisiae* (Prakash and Prakash, 2000). Specifically, Rad4 binds to the damage site and initiates sequential recruitment of NER factors such as TFIIH. The TFIIH multiprotein complex contains two DNA helicases, Rad3/XPD (yeast/human) and Ssl2/XPB (yeast/human), which function to unwind the damaged DNA during NER (Evans *et al.*, 1997; Prakash and Prakash, 2000). Helix opening by the actions of these two helicases is an essential step for the subsequent dual incision of the damaged DNA strand by Rad1-Rad10/XPF-ERCC1 and Rad2/XPG nucleases (Coin *et al.*, 2006; Oksenych and Coin, 2010). Our results raise the possibility that Hrq1 binds to the complex at the damaged site with the aid of Rad4 or other NER factor(s) and plays a redundant role in helix opening. Otherwise, Hrq1 may function in full separation of the damaged strand, based on a previous report that TFIIH-associated helicases catalyze initial formation of the open intermediate followed by full expansion of the DNA bubble around the damaged site promoted by additional NER factors such as XPA and RPA (Evans *et al.*, 1997; Compe and Egly, 2012). Purified Hrq1 protein did not unwind either double-stranded DNA or bubble structures (Kwon *et al.*, 2012). However, we cannot rule out the possibility that Hrq1 may be activated by NER factors to utilize double-stranded DNA as a substrate. Many NER factors are regulated by protein-protein interactions or protein modifications. For example, the ATPase activity of XPB helicase is regulated by XPC and other subunits of TFIIH (Compe and Egly, 2012). Thus, Hrq1 may require activating factor(s) to participate in NER. Identification of activating factors and/or NER factors that functionally interact with Hrq1 is necessary to determine the precise role of Hrq1.

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