

# Global Genome Nucleotide Excision Repair Proteins Rhp7p and Rhp41p Are Involved in Abasic Site Repair of *Schizosaccharomyces pombe*

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## Abstract

The roles of nucleotide excision repair (NER) proteins in removing UV-induced lesions are well defined. There are two distinct NER pathways: global genome NER (GG-NER) and transcription-coupled NER. In human GG-NER, two heteromeric protein complexes, DDB1-DDB2 and XPC-RAD23, are responsible for initial lesion recognition. Here, we examined the genetic interactions between GG-NER and base excision repair (BER) genes during abasic (AP) site repair of *Schizosaccharomyces pombe*. Mutants of *rhp7* (*rhp7-rhp16* are functional homologs of *DDB1-DDB2*) and *rhp41* (*XPC* homolog) were moderately sensitive to methyl methanesulfonate and slightly to sodium bisulfite. Nth1p most actively cleaves the AP site in *S. pombe*. Deletion of *rhp7* or *rhp41* from *nth1Δ* cells greatly increased their sensitivity to alkylation and deamination, indicating that Rhp7p and Rhp41p are involved in repair of the AP sites generated by the action of DNA glycosylase. Induction of *rhp7* and *rhp16* genes by different types of DNA damage supports the ability of GG-NER to remove non-bulky lesions. Therefore, GG-NER activity not only targets bulky DNA helix-distorting lesions, but can also efficiently remove AP sites synergistically with BER.

## Keywords

AP Site, Base Excision Repair, Global Genome Repair, Nucleotide Excision Repair, *Schizosaccharomyces pombe*

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## 1. Introduction

Abasic (AP) sites are one of the most common DNA lesions and can be generated by the spontaneous hydrolysis of the sugar base glycosidic linkage or by the action of monofunctional DNA glycosylases, removing damaged bases formed by deamination, oxidation and alkylation (reviewed in reference [1]). AP sites are potent blocks to replicative DNA polymerases, and translesion synthesis (TLS) for the AP-site bypass by specialized TLS DNA polymerases leads to high mutagenicity [2] [3]. RNA polymerase II can also be blocked at AP sites in the transcribed strand *in vitro* [4]. In many organisms, base excision repair (BER) is the major pathway for the repair of AP sites, and is initiated by cleavage of the phosphodiester bond either by AP endonuclease or the AP lyase activity of bifunctional DNA glycosylase [5] [6]. AP endonuclease hydrolyzes the 5' side of the AP site leaving a 3'-hydroxyl group and a 5'-deoxyribose phosphate. AP lyase cleaves 3' to the AP site leaving a 3'- $\alpha$ ,  $\beta$ -unsaturated aldehyde (3' block) and a 5'-phosphate. Following incision of the DNA backbone, 5'- or 3'-blocked ends are removed to allow repair DNA synthesis and ligation of the remaining nick. Although BER is an evolutionarily conserved process, a fission yeast *Schizosaccharomyces pombe* has some unique features of the pathway. AP sites are incised mainly by AP lyase activity of Nth1p, a sole bifunctional glycosylase in the yeast, and the major AP endonuclease Apn2p functions predominantly in removing the 3' block (Nth1p-dependent BER) [7]-[9].

Nucleotide excision repair (NER) is a versatile DNA repair pathway that removes a broad spectrum of DNA lesions that tend to distort the double helix, and is well known for its role in the repair of UV-induced cyclobutane pyrimidine dimers (CPD) and 6-4 pyrimidine-pyrimidone photoproducts (6-4PP) [1] [10] [11]. There are two distinct subpathways of NER: global genome NER (GG-NER) and transcription-coupled NER (TC-NER). GG-NER can repair lesions throughout the genome while TC-NER is specialized to correct lesions from the transcribed strand of active genes. In human GG-NER, the DDB1-DDB2 and XPC-RAD23B heteromeric protein complexes are responsible for initial lesion recognition, whereas the CSA and CSB proteins initiate the onset of TC-NER. Although the *S. pombe* NER pathway has not been extensively investigated, in the initial step of GG-NER, the Rhp7p-Rhp16p complex (functional homolog of human DDB1-DDB2) detects UV photolesions throughout the genome and transfers the lesion site to the Rhp41p-Rhp23p complex (XPC-RAD23B homolog) [12] [13]. The *Saccharomyces cerevisiae* *RAD7* and *RAD16* genes, homologs of *rhp7* and *rhp16*, respectively, are essential for GG-NER [14] [15]. Rad7p and Rad16p forms a protein complex with Abf1p and mediates UV-induced histone H3 acetylation which results in chromatin remodeling required for efficient GG-NER [16] [17]. *S. pombe* has two homologs of the human XPC, Rhp41p and Rhp42p; Rhp41p plays a major role in removing CPD in the transcribed and non-transcribed strands [18] [19]. *S. pombe* Rhp26p, a homolog of human CSB and budding yeast Rad26p, likely plays a key role in TC-NER [20] [21].

Although BER and NER are generally considered to remove different types of lesions, the disruption of both pathways of *S. cerevisiae* displays synergistic effects on damage sensitivity and genome stability in yeast cells, indicating a functional overlap between the two pathways [22]-[25]. *S. pombe* contains two paralogues of 3-methyladenine (3-meA) DNA glycosylase (Mag1p and Mag2p), and deficiency in both activities resulted in only a slight increase in sensitivity to methyl methanesulfonate (MMS), suggesting that Mag1p/Mag2p-initiated BER plays a minor role in repairing alkylation damage in yeast [26]-[28]. In contrast, *S. pombe* NER mutants *rad13* $\Delta$ , *rad16* $\Delta$ , and *swi10* $\Delta$  display substantial sensitivity to MMS [26] [28]-[30]. These genes encode structure-specific endonucleases required for dual incision of the damaged DNA strand, which is critical for both GG-NER and TC-NER. Further disruption of the BER gene (*mag1/mag2*, *nth1*, or *rad2*) from these NER mutants increases the spontaneous intrachromosomal recombinant frequency and sensitivity to MMS, suggesting the synergistic roles of BER and NER on repairing spontaneous and exogenous alkylation damage [26] [28]-[30]. Recently, we have shown that the *rhp41* and *rhp26* mutants exhibited sensitivity to MMS to the same extent as the *rad16* $\Delta$  strain [31]. Genetic interactions of *rhp41* and *rhp26* with BER genes showed the involvement of Rhp41p and Rhp26p in the TC-NER of MMS-damaged DNA. Given that the alkylated bases such as 3-meA are immediately removed to generate AP sites in BER-proficient cells, the function of NER is considered to be redundant in AP site repair, when considered with BER. In this study, we examined genetic interactions between NER and BER genes during repair of alkylation and deamination damage. The results showed that Rhp7p and Rhp41p, which generally function in early steps of GG-NER of UV photolesions, are involved in repairing AP sites generated by the action of DNA glycosylase.

## 2. Materials and Methods

### 2.1. Construction of Strains

Yeast strains used in this study are listed in **Table 1**. The *rhp7* $\Delta$  strain was provided by NBRP of MEXT, Japan. Double and triple mutants were constructed by targeted disruption of the NER gene *rhp7* and *rhp41* in BER-defective strains *mag1* $\Delta$ /*mag2* $\Delta$ , *nth1* $\Delta$ , and *apn2* $\Delta$  using a *kanMX6* marker [32]. The *rhp7::kanMX6* disruption cassette was prepared as follows. The genomic sequence of *rhp7* was amplified with the following primers: 5'-TCTCAAGGTATCAATGCTTCAGCAT-3' and 5'-TTATTGAACTTCACGCCCTATCAGA-3'. The gene fragment was then subcloned into pGEM-T vector (Promega, Fitchburg, Wisconsin, USA) by TA cloning. The *kanMX6* marker was amplified from pFA6a-*kanMX6* [32] by PCR and inserted between *HpaI* and *KpnI* sites of *rhp7* cloned in pGEM-T. The *rhp7::kanMX6* disruption cassette was amplified by PCR and used to transform the *nth1* $\Delta$  strain with lithium acetate [28]. The G418-resistant colonies were screened for the correct integration of *kanMX6* marker within the *rhp7* locus by PCR. The *rhp41::kanMX6* disruption cassette was prepared as previously described [31].

### 2.2. Survival Assay

Cells were grown overnight in YEA medium (0.5% yeast extract, 3% glucose, and 60  $\mu$ g/mL adenine) for survival assays. Cell density was determined by a Z1 particle counter (Beckman Coulter, Brea, CA, USA), and adjusted to  $1.0 \times 10^7$  cells/mL with sterilized water. The cells were diluted 10-fold serially from  $10^{-1}$  to  $10^{-3}$  in sterilized water. To measure the sensitivity of cells to chronic exposure to DNA damaging agents, dilutents (3  $\mu$ L) containing  $3 \times 10^1$  to  $3 \times 10^4$  cells were spotted onto YE medium containing MMS (Sigma-Aldrich, St. Louis, MO, USA) or sodium bisulfite (NaHSO<sub>3</sub>), and incubated for 3 days at 28°C. For the UV sensitivity test, serial dilutions were spotted onto YE medium, and irradiated with 254-nm UV light using a UV crosslinker (FS-1500; Funakoshi Inc., Tokyo, Japan) equipped with an internal photodetector for measuring the UV dose. Photographs of colonies were taken after 3 days of growth at 28°C.

### 2.3. Gene Expression Analysis by Reverse Transcription (RT)-PCR

Wild-type (WT) cells were cultured in YEA medium at 28°C until the optical density at 600 nm reached  $\sim 1.0$ . The cells were washed once with phosphate buffered saline (PBS), and resuspended in PBS at a cell density of  $2.0 \times 10^7$  cells/mL. A 10-mL portion of the cell suspension was poured into a 9-cm petri dish, and UV was irradiated at various dosages. When cells were exposed to MMS, the agent was added to the cell suspension at various concentrations. After incubation at 28°C for 20 min, MMS was neutralized with sodium thiosulfate. Damaged cells were washed once with YEA medium and incubated in medium at 28°C for 45 min with shaking. To extract total RNA, cells from the 10-mL culture were washed once with 10 mM Tris-HCl (pH 8.0) and 1mM EDTA, and resuspended in 0.2 mL of a buffer containing 1 M sorbitol, 0.1 M EDTA, 14 mM 2-mercaptoethanol,

**Table 1.** Strains used in this work.

Strains	Genotypes	Source
ED0665	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18</i>	ATCC No. 96993
FY18796	<i>h<sup>-</sup> ade6-M387 ura4-D18 rhp7::kanMX6</i>	YGRC <sup>a</sup>
KK05	<i>h<sup>+</sup> ade6-M216 leu1-32 ura4-D18 rhp41::kanMX6</i>	[31]
KK01	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 rad16::kanMX6</i>	[28]
HT02	<i>h<sup>-</sup> leu1-32 ura4-D18 mag1::ura4<sup>+</sup> mag2::ura4<sup>+</sup></i>	[28]
MS02	<i>h<sup>-</sup> leu1-32 ura4-D18 mag1::ura4<sup>+</sup> mag2::ura4<sup>+</sup> rhp7::kanMX6</i>	This study
TS01	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 nth1::ura4<sup>+</sup></i>	[8]
ES01	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 nth1::ura4<sup>+</sup> rhp7::kanMX6</i>	This study
ES02	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 nth1::ura4<sup>+</sup> rhp41::kanMX6</i>	This study
AY01	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 apn2::ura4<sup>+</sup></i>	[8]
ES01	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 apn2::ura4<sup>+</sup> rhp7::kanMX6</i>	This study
ST01	<i>h<sup>-</sup> ade6-M210 leu1-32 ura4-D18 apn2::ura4<sup>+</sup> rhp41::kanMX6</i>	This study

<sup>a</sup>YGRC: Yeast Genetic Resource Center.

and 4000 units/mL Zymolyase-20T. After incubation at 30°C for 30 min, total RNA was isolated using a reagent, ISOGEN II (Wako Pure Chemical Industries, Osaka, Japan), according to the manufacturer's instructions. RNAs were dissolved in RNase-free water, and treated with an amplification grade DNase I (Sigma-Aldrich). cDNA was prepared using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) with an oligo(dT)<sub>16</sub> primer. The target cDNA was amplified with GoTaq Green Master Mix (Promega) using the following gene-specific primer sets for *rhp7* (5'-GCAGGATCAAGTTTTACACTTTTATGCGG ATC-3' and 5'-GGGCAACAGTCAACTATAGCGTTGATAAC-3'), for *rhp16* (5'-GTTGTTGCTCCGGTTGT AGCAATTATGCA-3' and 5'-CCTTGATTTGATACCATGGGCCTCATCAA-3'), and for actin gene *act1* (5'-GCACCTGCCTTTTATGTTGCTATTCAAGC-3' and 5'-GTTGGAAAAGAGCTTCAGGGGCACG-3'). Cycle parameters were: denaturation, 94°C for 1 min; primer annealing, 51°C for 1 min; extension, 72°C for 20 sec with a first denaturation time of 5 min. Samples were electrophoresed on 2% agarose gels containing Gel Red Nucleic Acid Gel Stains (Biotium, Hayward, CA, USA) after 25 cycles of PCR. All cDNA samples were normalized to the expression of *act1*.

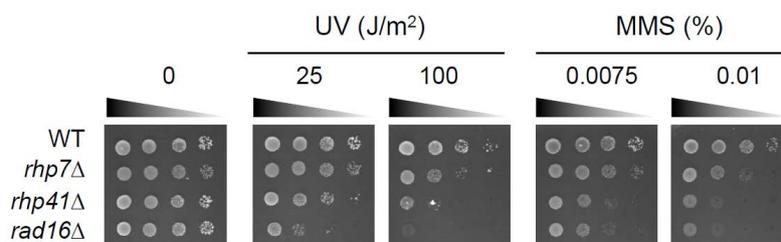
## 2.4. Promoter Analysis

An *rhp16* promoter-*lacZ* fusion plasmid was constructed by PCR amplifying a DNA fragment corresponding to positions -2185 to 958 (relative to the ATG start codon) using primers *rhp16*BamHI (5'-CTTGGATCCTGATT-CGGAGATCCATTCG-3') and *rhp16*PstI (5'-AACTGCAGAATGGGTTCTTTCATGTGATGG-3'). The resulting product was digested with *Bam*HI and *Pst*I before being ligated into the *Bam*HI and *Pst*I sites of pSPE-356 [33] to yield pSPE356-*rhp16*(-2185/958). The plasmid was introduced into WT ED0665 strain using the *S. pombe* Direct Transformation Kit (Wako). The cells harboring the reporter plasmid were cultured in SD medium (0.67% Difco yeast nitrogen base w/o amino acids, 3% glucose, 60 µg/mL adenine and 40 µg/mL leucine) at 28°C until the optical density at 600 nm reached ~1.0. The cells were washed once with PBS, and resuspended in PBS at a cell density of  $1.0 \times 10^7$  cells/mL. A 10-mL aliquot of the cell suspension was poured into a 9-cm petri dish, and the cells were irradiated with UV. When the cells were exposed to DNA-damaging agents, MMS or H<sub>2</sub>O<sub>2</sub> was added in cell suspension at appropriate concentrations and the cells were incubated at 28°C with shaking. The damaged cells were washed once with SD medium and incubated in medium at 28°C for 4 h with shaking.  $\beta$ -galactosidase activity in cells was measured by a liquid culture assay using *o*-nitrophenyl- $\beta$ -D-galactoside (ONPG) as substrate according to the user manual of Clontech Laboratories, Inc. (Yeast Protocol Handbook PT3024-1).

## 3. Results

### 3.1. Different Sensitivities of NER-Deficient Strains to UV and MMS Damage

UV and MMS sensitivities of *rhp7* $\Delta$  and *rhp41* $\Delta$  strains were compared to those of the WT strain and a NER endonuclease-deficient mutant *rad16* $\Delta$  (Figure 1). All NER mutants showed different levels of UV-sensitivity, even though *S. pombe* also uses an alternative mechanism, the Uve1p pathway, to remove UV damage [34]. Consistent with the literature [12] [19] [31], *rhp41* $\Delta$  and *rhp7* $\Delta$  were less sensitive to UV irradiation than *rad16* $\Delta$ , indicating that Rhp7p and Rhp41p are responsible for repairing some UV-induced lesions. *rhp7* $\Delta$  exhi-

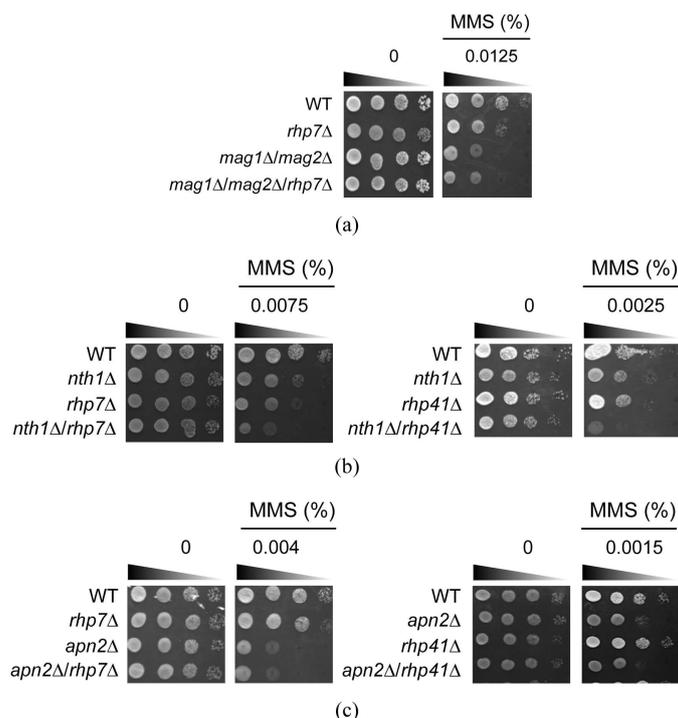


**Figure 1.** Sensitivity of various NER mutant strains to UV and MMS. WT cells and NER mutants (*rhp7* $\Delta$ , *rhp41* $\Delta$  and *rad16* $\Delta$ ) were grown overnight in YEA. Survival assays for UV and MMS were carried out as described in the Materials and Methods.

bited mild UV sensitivity among the NER mutants tested in this study. Although MMS damage is mainly repaired via the BER pathway in many organisms, several *S. pombe* NER mutants, including *rad16Δ* and *rhp41Δ*, have been shown to display hypersensitivity to MMS [26] [30] [31]. *rhp7Δ* was also sensitive to chronic exposure to MMS, indicating the contribution of Rhp7p to repair MMS-induced damage. *rhp41Δ* exhibited similar MMS sensitivity to *rad16Δ*, and *rhp7Δ* was more weakly sensitive to MMS. These results suggest that GG-NER proteins Rhp7p and Rhp41p are responsible, to different degrees, for the correction of MMS-induced DNA lesions.

### 3.2. Genetic Interactions of *rhp7* and *rhp41* with BER Genes during the Repair of Alkylated DNA

To study the genetic interactions between NER and BER genes during the repair of MMS-damaged DNA, double and triple mutants were generated for epistasis analysis. A harmful lesion 3-meA is removed by the DNA glycosylases Mag1p and Mag2p to initiate the BER pathway [27] [28]. Although a previous study demonstrated the synergistic interaction between *rhp41* and *mag1/mag2* genes [31], a triple mutant *mag1Δ/mag2Δ/rhp7Δ* exhibited the same sensitivity to MMS as the *mag1Δ/mag2Δ* mutant (Figure 2(a)). These results indicate the contribution of Rhp41p, but not Rhp7p, to correct 3-meA synergistically with Mag1p/Mag2p-initiated BER. Cytotoxic intermediates such as the AP site and the 3'-blocked end are generated during the BER of MMS-induced damage. In *S. pombe*, AP sites and 3'-blocked ends are thought to accumulate in the BER-deficient mutants *nth1Δ* and *apn2Δ*, respectively [9] [35]. Deletion of the *rhp7* or *rhp41* gene from the *nth1Δ* mutant greatly increased the sensitivity to MMS (Figure 2(b)), indicating that Rhp7p and Rhp41p are involved in repair of the AP site synergistically with Nth1p. However, a synergistic interaction was not observed between *apn2Δ* and NER mutants (Figure 2(c)). Thus, these NER proteins are unlikely to be responsible for repair of the 3'-blocked end.



**Figure 2.** Genetic interactions among BER and NER genes during repair of MMS-induced YEA damage. WT cells and various mutants were grown overnight in YEA. (a) Genetic interaction of *rhp7* with *mag1/mag2*. (b) Genetic interactions of *rhp7* and *rhp41* with *nth1*. (c) Genetic interactions of *rhp7* and *rhp41* with *apn2*. Survival assays for MMS at various concentrations were carried out as described in the Materials and Methods, and culture plates that clearly show the difference of viability of each strain were photographed.

### 3.3. Genetic Interactions among BER and NER Genes during Repair of Sodium Bisulfite-Induced Damage

Sodium bisulfite ( $\text{NaHSO}_3$ ) promotes the deamination of cytosine to uracil [36]. In *S. pombe*, the resultant uracil can be removed by a uracil DNA glycosylase Ung1p to initiate the BER mechanism [37] [38]. Although an *ung1* $\Delta$  mutant showed sensitivity to sodium bisulfite at the same level as the parental cells [38], the *nth1* $\Delta$  mutant was slightly sensitive probably due to the accumulation of uracil-derived AP sites (Figure 3). The *rhp41* $\Delta$  and *rhp7* $\Delta$  strains also displayed weak sensitivity to deamination. Double mutants *rhp7* $\Delta$ /*nth1* $\Delta$  and *rhp41* $\Delta$ /*nth1* $\Delta$  were more sensitive to sodium bisulfite than the corresponding single mutants, indicating a synergistic role of Rhp7p and Rhp41p to repair the AP site with Nth1p.

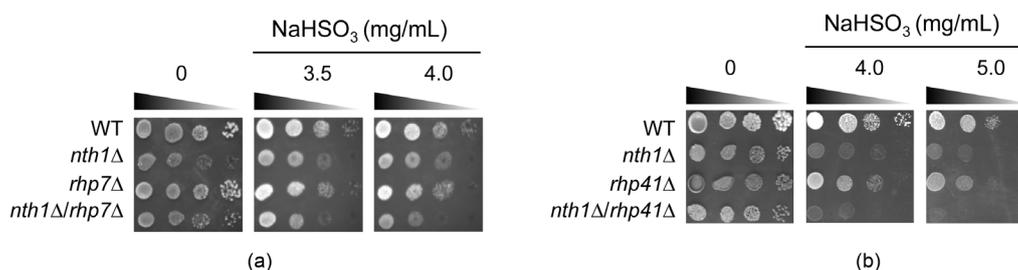
### 3.4. Gene Induction of *rhp7* and *rhp16* by DNA Damage

Rhp7p and Rhp16p form a protein complex and function in GG-NER [12]. Surprisingly, *rhp7* and *rhp16* genes are located very close together in a head-to-head orientation [12]. The expression of these genes in response to DNA damage was examined by quantitative RT-PCR using WT cells. Consistent with the literature [12], both *rhp7* and *rhp16* were significantly increased by UV irradiation ( $>50 \text{ J/m}^2$ ), although these genes were expressed at low levels under normal conditions (Figure 4(a)). Moreover, treatment with MMS at a high dose also led to a slight induction of these NER genes. DNA damage-dependent induction of these genes was further examined by a reporter assay using *rhp7* and *rhp16* promoter-*LacZ* fusion plasmids. The *S. pombe* data base (PomBase) shows that the transcription initiation site of each gene is mapped within the coding region of the opposite gene and that an *rhp16* antisense ncRNA is located just at the upstream region of the *rhp7* gene, implying the complicated regulation of *rhp7* and *rhp16* gene expression (Figure 4(b)). Although several fragments were tested as the promoter for *rhp7* transcription, gene induction by UV irradiation was not observed (data not shown). On the other hand, reporter activity of the *rhp16* promoter ( $-2185/958$ ) was induced about 4-fold by UV irradiation, and also weakly induced by MMS and hydrogen peroxide (Figure 4(c)). These results indicate that *rhp7* and *rhp16* are independently regulated and that the *rhp16* promoter is inducible by different types of DNA damage.

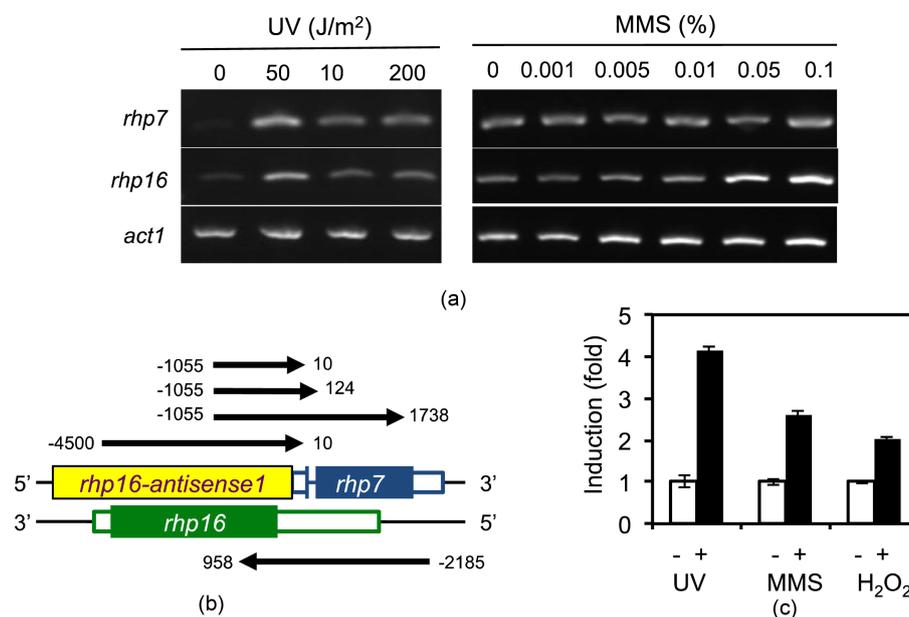
## 4. Discussion

NER is thought to be a versatile DNA repair pathway that removes bulky lesions that disrupt or distort the DNA double helix, such as CPD and (6-4)PP [1] [10] [11]. Recently, evidence has accumulated which shows that NER or NER proteins play significant roles in the repair of nonbulky lesions, such as the AP site and subtle base modifications, produced by oxidative and alkylating damage in mammalian and lower eukaryotic cells [9] [11] [13] [25] [39]. Here, we provide genetic evidence that GG-NER proteins Rhp7p and Rhp41p are involved in repairing AP sites synergistically with BER in *S. pombe* cells.

The mammalian XPC protein plays a key role in DNA damage recognition and the subsequent initiation of GG-NER [10]. Helix-distorting lesions are recognized by the XPC complex and repaired via GG-NER. On the other hand, XPC appears to be also involved in BER by interacting with various DNA glycosylases. XPC-HR23B functionally interacts with thymine DNA glycosylase (TDG) [40] and 3-meA DNA glycosylase [41] that facilitate BER of deamination and alkylation products, respectively. Although the XPC protein can recog-



**Figure 3.** Genetic interactions among BER and NER genes during repair of  $\text{NaHSO}_3$ -induced damage. WT cells and various mutants were grown overnight in YEA. (a) Genetic interactions of *rhp7* with *nth1*. (b) Genetic interactions of *rhp41* with *nth1*. Survival assays for  $\text{NaHSO}_3$  were carried out as described in the Materials and Methods.



**Figure 4.** DNA-damage dependent gene induction of *rhp7* and *rhp16*. (a) WT cells were exposed to UV irradiation (left panel) or MMS (right panel) at various doses indicated in panels, and then incubated at 28°C for 20 min. Extraction of total RNA and cDNA synthesis were carried out as described in the Materials and Methods. The expression of *rhp7* and *rhp16* gene was measured by PCR. All cDNA samples were normalized to the expression of *act1*. (b) Gene structure of *rhp7* and *rhp16* was illustrated with reference to the PomBase database (<http://www.pombase.org>). Four arrows over the map show the region used in the reporter assay for the *rhp7* promoter. Arrow under the map shows the region used for the *rhp16* promoter. Numbers on both sides of the arrows indicate the nucleotide number (relative to the ATG start codon of each gene) of the promoter fragments. (c) Induction of *rhp16* promoter activity by UV irradiation and chemical treatments. WT cells carrying the *rhp16* promoter-*lacZ* reporter were cultured at 28°C in SD medium until the cells proliferated exponentially. After damaging the cells, they were harvested and processed for liquid  $\beta$ -galactosidase assays. Results are the mean values (relative activity)  $\pm$  SD for three experiments.

nize and directly bind to AP sites, a protein-protein interaction seems to be important for the stimulation of DNA glycosylases by XPC, rather than competition for AP sites [42]. We showed that Rhp41p synergistically functions with Nth1p in repairing DNA alkylation and deamination in *S. pombe* cells (Figure 2 and Figure 3). Because elimination of the *rad16* gene encoding NER endonuclease from *nth1* $\Delta$  cells also displays an additive effect on sensitivity to MMS [31], Rhp41p possibly interacts with AP sites and leads to repair of the lesion via NER, rather than BER. In *S. cerevisiae*, NER competes with the actions of two AP endonucleases Apn1p and Apn2p in the removal of AP sites [24]. The potential competence of NER to target AP sites has been supported by an *in vitro* excision assay using a human cell-free extract [43].

The Rhp7p-Rhp16p complex has been demonstrated to be essential for GG-NER of UV photolesions by repair analyses with a nucleotide resolution [12]. Although no structural homologs of Rhp7p and Rhp16p have been identified in mammals, the Rhp7p-Rhp16p complex appears to share some striking functional similarities with DDB1-DDB2. A defect in *rhp7* increased sensitivity to different types of alkylating agents, MMS and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Figure 1) [13]. In *S. pombe*, an alkyltransferase-like protein (At1p) has the ability to bind DNA containing *O*<sup>6</sup>-methylguanine, and to protect against the deleterious effect of DNA alkylation damage by downstream recruitment of NER [13] [44]. In this case, the Rhp7p complex facilitates Rhp41p binding via an interaction with the At1p-lesion complex, possibly involving chromatin remodeling or displacement of At1p [13]. Here, we demonstrated an additive genetic interaction between *rhp7* and *nth1* during repair of DNA alkylation and deamination, indicating that the Rhp7p-Rhp16p complex is involved in repair of the AP site generated by the action of DNA glycosylase (Figure 2 and Figure 3). Induction of *rhp7* and *rhp16* genes by different types of DNA damage will support the ability to remove non-bulky lesions via GG-NER

(Figure 4). The human DDB1-DDB2 complex recognizes AP sites and mismatches as well as CPD and 6-4PP, although there are no reports on the function of DDB1-DDB2 in either BER or the mismatch repair pathway [45].

AP site repair is also accomplished by TC-NER, which is triggered when RNA polymerase II stalls at the lesion. Recent results showed that deletion of *S. pombe rhp26* (CSB homolog) in the *nth1*Δ strain greatly enhanced MMS sensitivity, suggesting that the AP site could be corrected by TC-NER [31]. In *S. cerevisiae*, the uracil-derived AP sites on the transcribed strand are also efficiently removed via TC-NER [25]. Therefore, both GG- and TC-NER activities not only target to bulky DNA helix-distorting lesions, but can also effectively remove AP sites and subtle base modifications.

## 5. Conclusion

We investigated the genetic interactions between NER and BER genes during repair of alkylation and deamination damage. Deletion of the *rhp7* or *rhp41* gene from the *nth1* mutant increased the sensitivity to MMS and NaHSO<sub>3</sub>, indicating that GG-NER proteins Rhp7p and Rhp41p are involved in repair of the AP sites generated by the action of DNA glycosylase. Furthermore, induction of *rhp7* and *rhp16* genes by different types of DNA damage supports the ability of GG-NER to remove non-bulky lesions. Therefore, GG-NER activity not only targets bulky DNA helix-distorting lesions, but can also efficiently remove AP sites synergistically with BER.

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