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Characterization of the *rhp7*$^+$ and *rhp16*$^+$ genes in *Schizosaccharomyces pombe*

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ABSTRACT

The global genome repair (GGR) subpathway of nucleotide excision repair (NER) is capable of removing lesions throughout the genome. In *Saccharomyces cerevisiae* the *RAD7* and *RAD16* genes are essential for GGR. Here we identify *rhp7*$^+$, the *RAD7* homolog in *Schizosaccharomyces pombe*. Surprisingly, *rhp7*$^+$ and the previously cloned *rhp16*$^+$ are located very close together and are transcribed in opposite directions. Upon UV irradiation both genes are induced, reaching a maximum level after 45–60 min. These observations suggest that the genes are co-regulated. *Schizosaccharomyces pombe rhp7* or *rhp16* deficient cells are, in contrast to *S.cerevisiae rad7* and *rad16* mutants, not sensitive to UV irradiation. In *S.pombe* an alternative repair mechanism, UV damage repair (UVDR), is capable of efficiently removing photolesions from DNA. In the absence of this UVDR pathway both *rhp7* and *rhp16* deficient cells display an enhanced UV sensitivity. Epistatic analyses show that *rhp7*$^+$ and *rhp16*$^+$ are only involved in NER. Repair analyses at nucleotide resolution demonstrate that both Rhp7 and Rhp16, probably acting in a complex, are essential for GGR in *S.pombe*.

INTRODUCTION

During evolution various repair mechanisms have developed to deal with the deleterious effects of DNA damaging agents (reviewed in 1). Nucleotide excision repair (NER) is a versatile mechanism capable of removing a large variety of lesions from the genome including UV-induced cyclobutane pyrimidine dimers (CPDs). The eukaryotic NER reaction has been reconstituted *in vitro* using repair components from *Saccharomyces cerevisiae* or human cells which allowed identification of a set of proteins, necessary and sufficient (referred to as core NER proteins), to perform the reaction on plasmid DNA. NER is capable of removing lesions throughout the genome; however, there is an intra-genomic heterogeneity in the efficiency of repair. It was shown that transcriptionally active loci are repaired faster than the bulk of the genome (2). This enhanced repair that results from the increased rate of repair of lesions from the transcribed strand, is dependent on ongoing transcription (3,4) and is therefore called transcription-coupled repair (TCR). This phenomenon is found in organisms ranging from *Escherichia coli* to humans (5–8). Lesions in non-transcribed DNA are obviously not removed by TCR but are nevertheless repaired by NER. This process is called global genome repair (GGR), and removes lesions from the genome overall. The relative efficiencies of these NER subpathways determine the difference in kinetics of CPD removal from both strands resulting in differential repair (reviewed in 9). In general, TCR is fast whereas GGR is slower. Besides the core NER proteins essential for all NER, specific proteins have been identified that are exclusively devoted to either TCR or GGR indicating that both subpathways of NER are genetically distinctive. Both the *S.cerevisiae* Rad26 and the human CSA and CSB gene products are specifically involved in TCR (10,11), while the *S.cerevisiae* Rad7 and Rad16 and the human XPC proteins are essential for GGR (12,13).

Saccharomyces cerevisiae rad7 and *rad16* mutants were shown to be deficient in removal of CPDs from the silent mating-type loci (14), non-transcribed strands (12) and promoter regions (15). Survival experiments demonstrated that, as the result of a partial NER defect, *rad7* and *rad16* mutants are intermediately sensitive to UV light (16,17). Genetic and biochemical data indicated that both proteins are functional as a complex (12,18,19) and are likely to be involved in damage recognition, although recently a requirement in post-incision events was proposed (20). Previously we identified *rhp16*$^+$, the *Schizosaccharomyces pombe* homolog of *RAD16* (21). In contrast to *S.cerevisiae rad16* mutants, *rhp16* deficient cells are not sensitive to UV light. Besides NER, *S.pombe* also uses a second repair mechanism, UV damage repair (UVDR), to remove photolesions from DNA (22). The *uvde*$^+$ gene, coding for the enzyme performing the first step in the UVDR pathway (23), has recently been cloned (24) and homologs of this gene are present in *Neurospora crassa* (25) and *Bacillus subtilis* (24). The Uvde protein is able to recognize photolesions and incise the DNA 5' of the lesion (24). The UVDR pathway in *S.pombe* operates with the same efficiency on both the transcribed and non-transcribed strands (TS and NTS respectively). In contrast, CPDs are removed rapidly from the TS and very slowly from the NTS by NER (7). Since the UVDR pathway is very efficient, *rhp16* deficient cells show the same sensitivity as NER proficient cells to UV light. However *rhp16* deficient cells are mildly

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Table 1. The *S.pombe* strains used in this study

Strain	Genotype[a]	Source
Y4	*h*$^+$ *ade6*-M216 *leu1-32 ura4*-D18	A. Yasui
Y21	*rad13*::*ura4*$^+$	A. Yasui
Y26	*rad13*::*ura4*$^+$ *uvde*::LEU2	A. Yasui
Y48	*h*$^-$ *uvde*::LEU2	A. Yasui
Y53	*h*$^+$ *uvde*::LEU2	A. Yasui
MGSP44	*rhp7*::hisG	This study
MGSP50	*rhp16*::hisG	This study
MGSP52	*rhp7*::hisG *rhp16*::hisG-*ura4*$^+$-hisG	This study
MGSP45	*h*? *uvde*::LEU2 *rhp7*::hisG-*ura4*$^+$-hisG	This study
MGSP48	*h*? *uvde*::LEU2 *rhp16*::hisG-*ura4*$^+$-hisG	This study
MGSP63	*h*? *uvde*::LEU2 *rhp7*::hisG *rhp16*::hisG-*ura4*$^+$-hisG	This study
MGSP64	*rad13*::*ura4*$^+$ *rhp7*::hisG	This study
MGSP51	*rad13*::*ura4*$^+$ *rhp16*::hisG	This study

[a]All strains are isogenic and the remainder of the genotype conforms Y4.

sensitive towards cisplatin (21), which induces lesions that are not repaired by the UVDR mechanism. This suggests a function of Rhp16 in NER. The UVDR pathway is abrogated in *uvde* deficient cells and the role of Rhp16 in DNA repair in *S.pombe* can be studied in this background, where CPDs are exclusively removed by NER.

Until now no homolog of the *RAD7* gene was found in *S.pombe*. Here we report the identification of the *rhp7*$^+$ gene, the *RAD7* homolog in *S.pombe*. Based on the homology between Rhp7, Rad7 and the porcine RNase inhibitor (RI), we speculate on the tertiary structure of Rhp7 and Rad7. Furthermore, we show that *uvde rhp7* and *uvde rhp16* double mutants are intermediately sensitive to UV light, suggesting a partial NER defect. Repair analyses at nucleotide resolution in the *sprpb2*$^+$ gene confirm that the NTS is not repaired at all in these double mutants, indicating the existence of GGR in *S.pombe* and that Rhp7 and Rhp16 are essential for this subpathway of NER.

MATERIALS AND METHODS

Screening the databases

The *S.pombe* genomic sequence database, produced by the *S.pombe* Sequencing Group at the Sanger Centre, was searched using the tblastn program (version 2.0a) (26). The *rph7*$^+$ DNA sequence data were retrieved from ftp://ftp.sanger.ac.uk/pub/yeast/sequences/pombe . The EMBL accession number of Rhp7 is CAA20907. The alignment was made by the ClustalW program version 1.74 (27) using the blosum option as weight matrix. Alignments were fine-tuned manually.

Strains and media

Schizosaccharomyces pombe strains were maintained on complete medium (YES; 5 g/l yeast extract, 30 g/l glucose, 2% bacto agar supplemented with 75 mg/l adenine and 75 mg/l uracil). Cells were grown in liquid complete (YES) medium at 29ºC under vigorous shaking. Complementation experiments in *S.cerevisiae* were carried out on YEPD (1% yeast extract, 2% bacto peptone, 2% glucose) and YEPG plates (1% yeast extract, 2% bacto peptone, 2% galactose).

The *S.pombe* strains used in this study are mentioned in Table 1. Strains MGSP44, MGSP50, MGSP51, MGSP52 and MGSP64 were created by one-step gene replacement using a LiAc-based transformation method (28); strains MGSP45, MGSP48 and MGSP63 result from crosses (29) between isogenic backgrounds. The *rad13*::*ura4*$^+$ disruption construct was a gift of Drs A. Yasui and S. Yasuhira (Tohoku University, Japan).

Cloning of *rhp7*$^+$

Two PCR primers, rhp7start (5'-TTG GGG ATC CAA ATG TCA AGT GGA AGT CGG GTG) and rhp7stop (5'-TTA GAT CTG CAG TTA ACC GGT TTG AAC TTC ACG CCC TAT CAG AAG), were designed to amplify the *rhp7*$^+$ gene (*rhp7*$^+$ sequence underlined). The *rhp7*$^+$ DNA PCR products were digested with *Bam*HI–*Pst*I and cloned in the *Bam*HI and *Pst*I sites of pUC19. A *Sal*I–*Xba*I fragment from the resulting plasmid was replaced by the hisG-*ura4*$^+$-hisG cassette [a gift of Dr McNabb, Massachusetts Institute of Technology, Cambridge, MA (30)] to give rise to pUCrhp7::hisG-*ura4*$^+$-hisG, which was used to create *rhp7* deletion strains.

rhp7$^+$ cDNA was made using oligo(dT)$_{25}$ beads (Dynal), according to the manufacturer's instructions. A cDNA PCR product (digested with *Bam*HI and *Pst*I) was ligated in the *Bam*HI and *Pst*I sites of pUC18 and sequenced. From this plasmid, *rhp7*$^+$ was cloned in pYET2 (a pYES2 derivative where an *Acc*I fragment is replaced by the *TRP1* selection marker) resulting in pYETrhp7.

RNA induction

Wild-type *S.pombe* cells (strain Sp.972 h⁻), grown to an optical density of 1.0, were irradiated with 100 J/m² UV light (254 nm) in phosphate-buffered saline (PBS) and transferred to liquid YES medium. Samples were taken 0, 7.5, 15, 30, 45, 60, 90, 120 and 150 min after irradiation and RNA was isolated using standard techniques. An aliquot of 20 µg of total RNA was used for northern blotting. Blots were hybridized with *rhp7*⁺, *rhp16*⁺ or *ura4*⁺ specific probes. The *ura4*⁺ signal was used as an internal standard.

Survival experiments

Cells were grown to an optical density of 1.0. Serial dilutions of these cells were prepared in PBS and spread on YES plates. The plates were irradiated with increasing doses of UV light and incubated at 29°C. After 4 days, colonies were counted and survival was calculated.

Two-hybrid system

DNA fragments containing the complete sequences of the *RAD7*, *RAD16*, *rhp7*⁺ (without intron) and *rhp16*⁺ were cloned in frame with the GAL4-DNA binding domain and the GAL4-transcription activating domain in the two-hybrid plasmids pGBT9 and pGAD424 (Clonetech). Interaction was tested in the *S.cerevisiae* strain PJ69-4A [a gift of Dr P. James, University of Wisconsin, Madison, WI (31)] by co-transformation pGBTrhp7 and pGADrhp16 as well as pGBTrad16 and pGADrad7 and selecting for histidine phrototrophy.

UV irradiation and DNA isolation

Yeast cells diluted in chilled PBS were irradiated with 40 J/m² UV light (254 nm) at a rate of 3.5 J/m²/s (Philips TUV 30W). Cells were collected by centrifugation, resuspended in complete medium and incubated for various times at 29°C prior to DNA isolation (32). DNA samples were purified on CsCl gradients (33). Repair analysis at nucleotide resolution in the *sprpb2*⁺ gene are described extensively elsewhere (7,15).

RESULTS

Identification of Rhp7 and domain structure

Searching the *S.pombe* sequence database at the Sanger Centre using the tblastn program (26) revealed an open reading frame on chromosome 3, which we designated *rhp7*⁺, showing homology to the *S.cerevisiae* Rad7 protein sequence (Fig. 1A; EMBL accession number CAA20907). The putative *S.pombe* Rhp7 protein consists of 563 amino acids, a size very similar to the *S.cerevisiae* Rad7 protein (565 amino acids). In the predicted Rhp7 protein a C4 type zinc-finger motif is identified at position 125–152 that is not present in the Rad7 protein (Fig. 1A).

Previously, five leucine-rich repeats (LRRs) were identified in the Rad7 protein sequence (34). The alignment of Rhp7 and Rad7 allowed us to identify 14 such repeats in the C-terminal two-thirds of the proteins (Fig. 1A). The alignment and the consensus sequence of the 14 LRRs of Rhp7 are given in Figure 1B. These leucine-rich motifs are found in many proteins. The porcine RI contains 15 LRRs which give the protein a very distinct 'horseshoe-like' appearance (34). The presence of 14 of these repeats in Rhp7 and Rad7 suggests a structural homology with RI. In RI, each LRR is composed of a β-sheet and an α-helix connected by a turn (35). Each repeat is stabilized because sidechains of hydrophobic residues of the β-sheet and α-helix protrude and form a hydrophobic core. Furthermore, β-sheets tend to pack against the helices that precede. The arrangement of the β–α structural units results in the 'horse-shoe-like' shape of the protein, with the α-helices on the outside and the β-sheets aligned on the inner circumference (35). Here we use the structure of RI to model part of the Rad7 and Rhp7 proteins using Swiss Model (36). Conserved leucine residues forming the β-sheet of the LRRs in RI were used as 14 anchor points in the alignment of RI and Rhp7. Hydrophobic residues in the α-helices of RI were aligned with homologous residues in Rhp7. Variations in length of the connection between the β-sheets and α-helices are common in this motif (34). The overall similarity at conserved positions between Rhp7 and RI is >80%. The homology modeling of the C-terminal two-thirds of Rhp7 resulted in a putative model given in Figure 1C.

The promoter of *rhp7*⁺

A putative TATA box is found from –50 to –59 (relative to the first nucleotide of the putative start codon ATG = +1). Consensus intron border sequences [GTANGN$_{(21–101)}$CTPuAN$_{(4–17)}$AG] (37) were identified near the start codon and cDNA sequencing confirmed the presence of an intron from +58 to +105. A putative damage responsive element (DRE) box is identified at position –154 to –163, showing homology with DRE boxes found in promoters of the *S.pombe* damage inducible genes *rhp51*⁺ (38) and *uvde*⁺ (39), and with DRE boxes found in various *S.cerevisiae* genes [consensus sequence C(T/G)(T/A)GG(T/A)NT(T/C)(A/C)]. Surprisingly, *rhp7*⁺ maps very close to the *rhp16*⁺ gene, and is transcribed in the opposite direction. The Rhp7 and Rhp16 proteins are involved in the same DNA repair process, suggesting the possibility of a shared regulatory element within the 405 nucleotides separating the start codons of these genes.

Induction of *rhp7*⁺ RNA by UV irradiation

To study a possible co-regulation of the *rhp7*⁺ and *rhp16*⁺ genes, *S.pombe* cells were irradiated with UV light and *rhp7*⁺ RNA levels were compared with *rhp16*⁺ RNA levels, which we have shown to be elevated following UV irradiation (21). The northern blots in Figure 2A demonstrate that *rhp7*⁺ RNA levels are elevated after irradiation with 100 J/m² UV light. The kinetics of induction appear similar for *rhp7*⁺ and *rhp16*⁺ and for both genes the maximum induction is reached 45–60 min after UV irradiation. The induction factor of *rhp7*⁺ RNA is 15–20 times, *rhp16*⁺ is induced 7–10 times (Fig. 2A) (21). The basal level of transcription of *rhp7*⁺ and *rhp16*⁺ seems to be very low compared to the expression of the *ura4*⁺ gene.

Intermediate UV sensitivity of *rhp7* and *rhp16* mutants in the absence of the UVDR pathway

Disruption of *rhp7*⁺ and *rhp16*⁺ were created in various isogenic backgrounds (Table 1) and the UV survival was determined. (Fig. 2B and C). Repair proficient, *rhp7* and *rhp16* deficient cells all have the same UV sensitivity due to the efficient UVDR pathway. Disruption of the *rhp7*⁺ or *rhp16*⁺ genes in *uvde* deficient cells reduces the survival. Disruption of both *rhp7*⁺ and *rhp6*⁺ in a *uvde* deficient background does not lead to a further enhancement of the UV sensitivity, indicating that Rhp7 and Rhp16 act in the same pathway. In an NER deficient (*rad13*) background, disruption of *rhp7*⁺ or *rhp16*⁺ does not

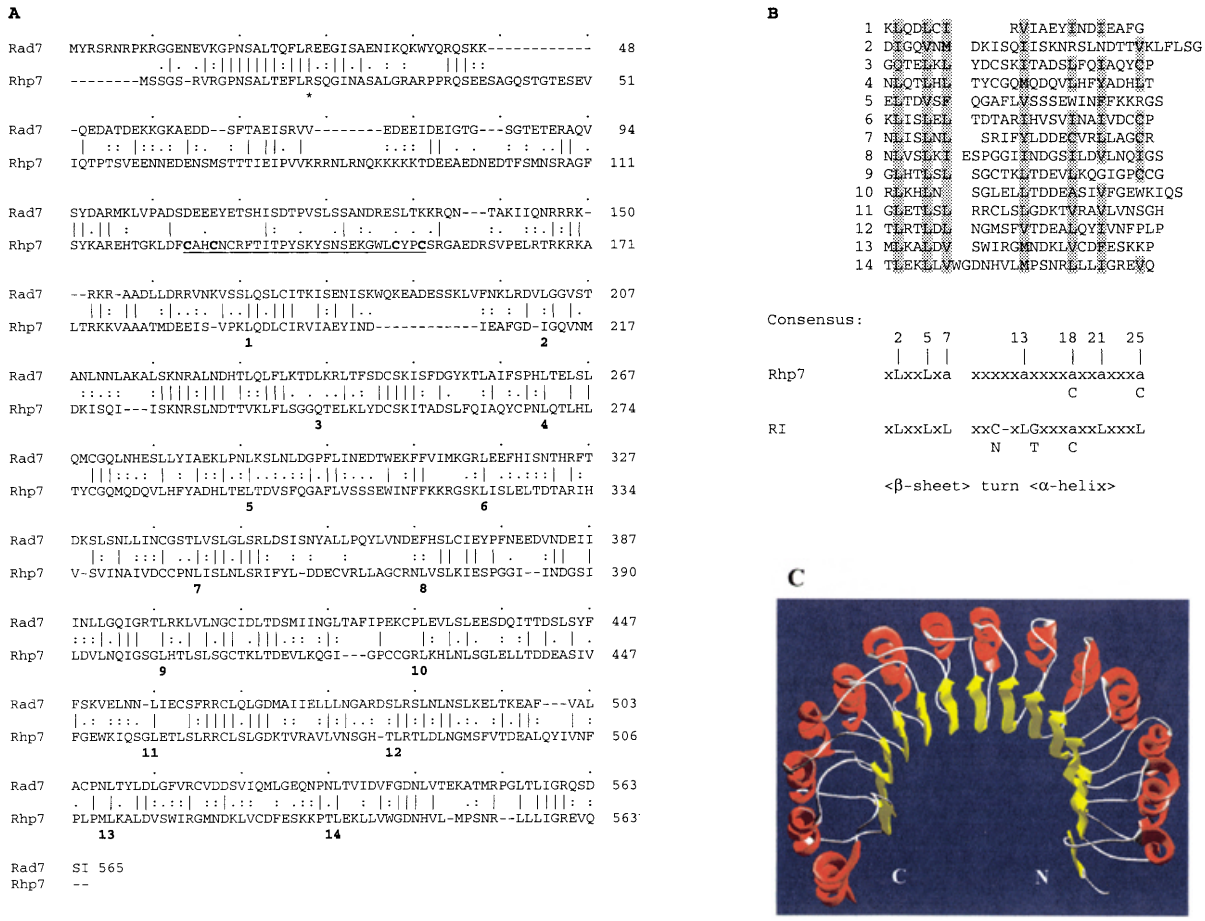


Figure 1. (**A**) The alignment of the Rad7 and the Rhp7 protein sequences. Rhp7 and Rad7 were aligned using the ClustalW program version 1.74 (27). Identical residues are indicated by vertical lines; degrees of similarity are indicated by colons and full-points. The position of the intron (between residue R-19 and S-20) is marked with an asterisk. The putative C4 zinc-finger domain is underlined and the cysteine residues are boldfaced. The LRRs are numbered 1–14. Overall, there is 31% identity and 40% similarity between Rhp7 and Rad7. (**B**) Alignment of the LRRs. The 14 LRRs of Rhp7 are aligned and conserved residues are shaded. The alignment results in the consensus sequence given. For comparison the consensus sequence of the LRRs of the RI is given. (a indicates one of the following residues: V, I, L, M, F, Y or A; x denotes any amino acid.) (**C**) The putative structure of Rhp7 based on the LRRs of the RI. β-Sheets are depicted in yellow, α-helices are red. Both N- and C-termini are indicated.

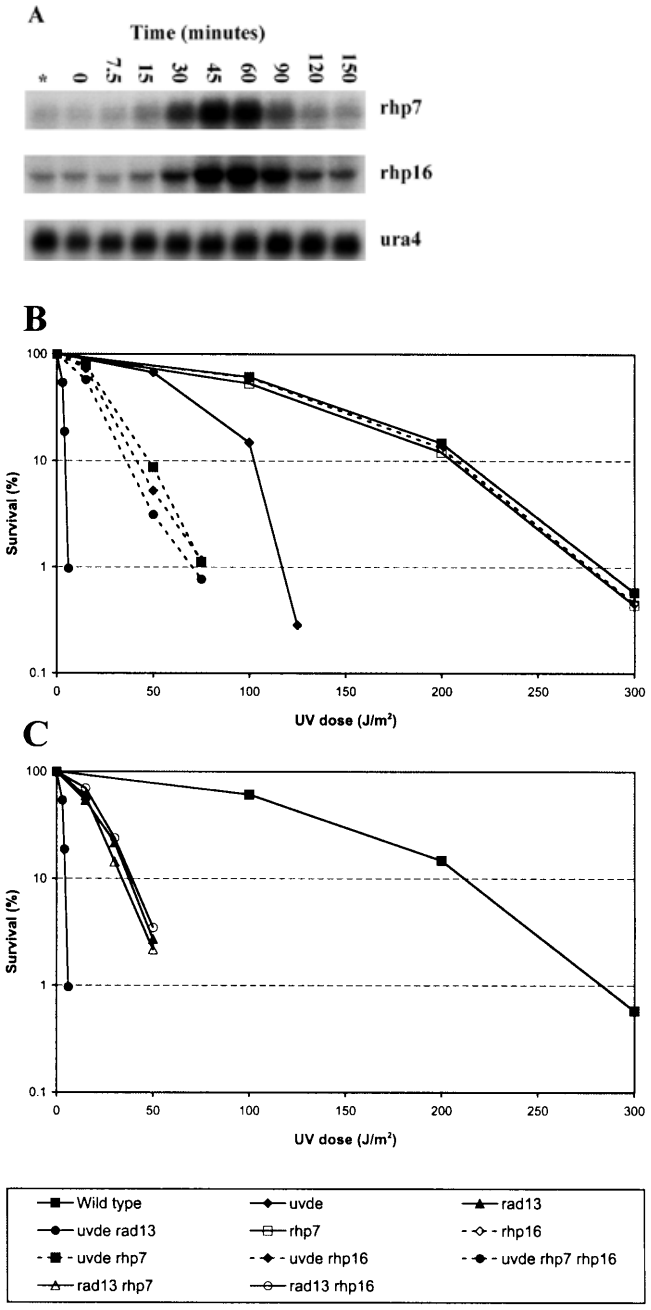
lead to an enhancement of the UV sensitivity, indicating that Rhp7 and Rhp16 are solely involved in NER.

Interaction of Rhp7 and Rhp16

The UV-sensitivity analyses described above demonstrate that Rhp7 and Rhp16 function in the same pathway, possibly as part of the same complex. The yeast two-hybrid system was used to detect possible interactions between the *S.pombe* Rhp7 and Rhp16 proteins and to confirm the previously reported *S.cerevisiae* Rad7 and Rad16 interaction (40). Expression plasmids coding for fusion proteins between the Gal4-DNA binding domain (Gal4db) or the Gal4 transcription activating domain (Gal4ta) with Rhp7, Rhp16, Rad7 and Rad16 were introduced to strain PJ96-4A (31) where transcription of the *HIS3* gene depends on the interaction of the fusion proteins. Co-transformants are analyzed for histidine phototrophy. The combinations Rhp7 with Rhp16 and Rad7 with Rad16 are histidine prototroph indicating that Rhp7 and Rhp16 as well as Rad7 and Rad16 do interact (data not shown). Neither Rhp7 and Rad16 nor Rhp16 and Rad7 interact in this two-hybrid assay (data not shown).

The UV sensitivity of *S.cerevisiae rad7* deficient cells is not complemented by rhp7$^+$

The plasmids pYETrhp7 and pYESrhp16 (21) were transformed to *S.cerevisiae rad7* and *rad16* disruptants respectively to study cross-complementation. In these plasmids expression of *rhp7*$^+$ and *rhp16*$^+$ is driven by the galactose-inducible Gal1 promoter. In Figure 2D, various strains are tested for UV survival on a galactose-containing plate. *Saccharomyces cerevisiae rad7* (lane 2) and *rad16* (data not shown) mutants are sensitive to UV light. The presence of empty vectors in *rad7* and *rad16* deficient cells has no effect on survival (lanes 3 and 5). pYETrhp7 is unable to rescue the UV phenotype of *rad7* deficient *S.cerevisiae* cells (lane 4). In contrast, pYESrhp16 does result



in a (partial) complementation of the sensitivity of *rad16* deficient *S.cerevisiae* cells (compare lanes 5 and 6). Introduction of pYETrhp7 and pYESrhp16 in *S.cerevisiae rad7 rad16* double mutants does not result in a complementation of the UV sensitivity (lane 7).

Repair of transcribed DNA is not dependent on Rhp7 or Rhp16

The function of Rhp7 and Rhp16 in repair was studied at nucleotide level in absence of the UVDR pathway, using a method we described previously (7,15). Repair of both the TS and the NTS of the *sprpb2*$^+$ gene was analyzed. Figure 3A shows repair of the TS of the *sprpb2*$^+$ gene of *uvde* deficient cells. A 532 bp *Eco*RI fragment of the *sprpb2*$^+$ gene was isolated from *S.pombe uvde* deficient cells after various incubation times post-UV irradiation and subsequently treated with the T4EndoV enzyme to visualize remaining CPDs. The initial lesion distribution is shown in Figure 3A, lane 2 (t = 0 min). The time-dependent decrease in the intensity of the bands indicates repair (lanes 3–5). The repair of CPDs from the TS of the *sprpb2*$^+$ gene in a *uvde rhp7* double mutant is given in Figure 3B. The rate of lesion removal is, after correction for loading variations, the same as in *uvde* deficient cells (compare Fig. 3A and B). Likewise, *uvde rhp16* deficient cells show the same repair rate as *uvde* deficient cells (compare Fig. 3A and C). In the absence of both NER and UVDR there is no repair of lesions detectable within the time of the experiment (Fig. 3D).

Rhp7 and Rhp16 are essential for GGR

The repair analysis of the NTS of the *sprpb2*$^+$ gene in *uvde* deficient cells demonstrates that there is repair, although much slower than repair of CPDs from the TS (7) (compare Fig. 3A and E). This difference in repair rate suggests that, in *S.pombe*, TCR is much more rapid than GGR in removing photolesions from DNA. Repair analyses of *uvde rhp7* and *uvde rhp16* deficient cells show a complete abrogation of lesion removal from the NTS (Fig. 3F and G), indicating that both Rhp7 and Rhp16 are essential for repair of CPDs from the NTS.

Figure 2. (**A**) Induction of *rhp7*$^+$ and *rhp16*$^+$ RNA after UV irradiation. Autoradiogram of a northern blot probed for *rhp7*$^+$ (upper panel), *rhp16*$^+$ (middle panel) and *ura4*$^+$ (lower panel). RNA was isolated at timepoints indicated post-UV irradiation. In the lane marked with an asterisk, RNA from non-irradiated cells was used to indicate the basal level of expression. The *ura4*$^+$ signal was used as an internal standard. (**B** and **C**) Survival curves of different *S.pombe* strains. Plated yeast cells on plates were irradiated with the indicated doses of UV light and, after incubation at 29°C for 4 days, colonies were counted and survival was calculated. The effect of disruption of *rhp7*$^+$ and *rhp16*$^+$ on survival is only seen in *uvde* deficient cells (B) and not in repair proficient (B) nor in *rad13* deficient cells (C). (**D**) *Schizosaccharomyces pombe rhp7*$^+$ does not complement the UV sensitivity of *S.cerevisiae rad7* deficient cells. *Saccharomyces cerevisiae rad7* deficient cells transformed with *rhp7*$^+$ expression (lane 4) or control (lane 3) plasmids were irradiated with the UV dose indicated. Also, *rad16* deficient cells transformed with *rhp16*$^+$ expression (lane 6) and control (lane 5) plasmids, and *rad7 rad16* deficient cells transformed with *rhp7*$^+$ and *rhp16*$^+$ expression plasmids (lane 7), were irradiated. Survival was compared with repair proficient (lane 1) and *rad7* deficient *S.cerevisiae* cells (lane 2). Expression of *rhp7*$^+$ and *rhp16*$^+$ was induced with galactose. The plate was incubated at 28°C for 3 days.

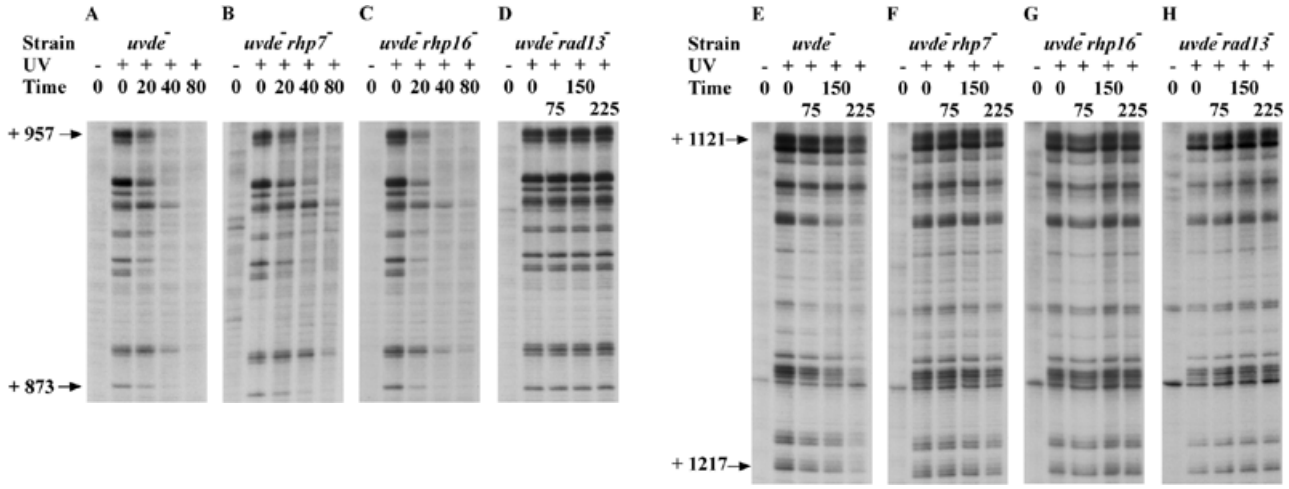


Figure 3. Repair of CPDs from the transcribed and the non-transcribed strands of the *S.pombe sprpb2*$^+$ locus. Cells were irradiated with 40 J/m^2 UV light and allowed to repair DNA for the time periods indicated. DNA was isolated and the fragment of interest was labeled and treated with the CPD-specific enzyme T4EndoV. Lesion-specific bands in the TS [(A–D) position +957 to +873] and in the NTS [(E–H) position +1121 to +1217 relative to the start codon ATG designated +1] of *uvde* (**A** and **E**), *uvde rhp7* (**B** and **F**), *uvde rhp16* (**C** and **G**) and *uvde rad13* (**D** and **H**) deficient cells are shown in the autoradiograms. Lanes indicated UV− contain non-irradiated DNA treated with T4EndoV. The initial lesion distribution is given at 0 min. There is a rapid time-dependent decrease in the intensity of the bands in (A)–(C) indicating rapid repair. The time-dependent decrease in the intensity of the bands in (E) indicates slow repair. In (D), (F), (G) and (H) no CPDs are repaired.

DISCUSSION

An open reading frame, homologous to the *S.cerevisiae RAD7* gene, was identified from the *S.pombe* genomic sequence database. We cloned the gene and designate it *rhp7*$^+$. The *rhp7*$^+$ and *rhp16*$^+$ genes were disrupted in various genetic backgrounds to study their function in DNA repair. We demonstrate that Rhp7 and Rhp16 are exclusively involved in NER. In the absence of the UVDR mechanism, disruption of *rhp7*$^+$ and *rhp16*$^+$ results in an intermediate UV sensitivity because of a partial NER defect. Both Rhp7 and Rhp16, like their *S.cerevisiae* homologs, are essential for GGR and probably are part of one protein complex. These observations clearly demonstrate that *rhp7*$^+$ and *rhp16*$^+$ are the structural and functional homologs of *RAD7* and *RAD16*.

Although the N-terminal parts of the Rhp7 and Rad7 protein sequences are diverged and a putative C4 zinc-finger domain is identified in Rhp7 that is not present in Rad7, the structure of the C-terminal two-thirds of both proteins is very similar. Homology modeling, based on the presence of 14 LRRs in Rhp7 and Rad7, suggests that part of both proteins might have a similar 'horseshoe-like' structure as the porcine RI (35). RI strongly binds RNase A on the inside of the 'horseshoe' (41). This suggests that also (some) interactions of Rad7 or Rhp7 are mediated this way. Indeed, mutations in RAD7 that abolish the reported interaction with Sir3 (42) locate either in residues that form the inner surface of the Rhp7/Rad7 structure or in conserved hydrophobic residues (unpublished observations).

The *S.cerevisiae RAD7* and *RAD16* genes locate on different chromosomes. The *rhp7*$^+$ and *rhp16*$^+$ genes, however, map very close together and are transcribed in opposite directions with only 405 nucleotides between both start codons, suggesting a co-regulation. Indeed, upon UV irradiation both *rhp16*$^+$ and *rhp7*$^+$ are induced at the RNA level and both reach the peak of induction after 45–60 min. Furthermore, a putative DRE box is identified, resembling those found in promoters of other damage inducible genes like *rhp51*$^+$ (38) and *uvde*$^+$ (39), suggesting the involvement of this element in the induction of both *rhp7*$^+$ and *rhp16*$^+$ RNA.

The *rhp16*$^+$ gene is able to complement the UV sensitivity of *S.cerevisiae rad16* deficient cells (21) (Fig. 2D). However, introducing *rhp7*$^+$ cDNA in *rad7* deficient *S.cerevisiae* cells does not result in a rescue of the UV sensitivity. Possibly Rhp7 is unable to form a functional complex with Rad16. Rhp7 and Rhp16 are able to form a complex in *S.cerevisiae* as demonstrated in the two-hybrid assay. Therefore, we co-expressed *rhp7*$^+$ and *rhp16*$^+$ in *S.cerevisiae rad7 rad16* deficient cells and examined complementation of the UV sensitivity by the *S.pombe* Rhp7–Rhp16 complex. However, no complementation was detected. These observations suggest that the Rhp7–Rhp16 complex cannot interact with a third factor in *S.cerevisiae* and that this interaction normally is mediated by Rad7, most likely via the N-terminal part, because this is the part where the Rhp7 and Rad7 sequences diverge the most.

We showed previously that in the absence of UVDR, lesions from the transcribed DNA are removed very rapidly while lesions from the NTS are repaired more slowly (7). Here we show that Rhp7 and Rhp16 are essential for removal of lesions from non-transcribed DNA by NER. This indicates that GGR is conserved in *S.pombe* and that Rhp7 and Rhp16 are the genetic determinants of this subpathway, like the *S.cerevisiae* homologs.

The question now arises whether homologs of Rhp7 and Rhp16 exist in higher eukaryotes. In the *Caenorhabditis elegans* genome, no sequence homologs of Rhp7 or Rhp16 are identified. Also, no homologs were identified from human databases, suggesting that no sequence homologs exist beyond *S.pombe*.

DDB, a heterodimer of p48 and p127 present in human cells (43), shares at least some of the properties of Rhp7–Rhp16 and Rad7–Rad16. DDB was identified by its capacity to bind strongly to damaged DNA and, recently, Hwang *et al.* suggested that DDB plays an important role in targeting CPDs for GGR (44). Although the sequences of Rhp7 and Rhp16 do not resemble p48 or p127, DDB might be the functional homolog in mammalian cells and possibly in all higher eukaryotes.

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