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The *Schizosaccharomyces pombe rhp3*$^+$ gene required for DNA repair and cell viability is functionally interchangeable with the *RAD3* gene of *Saccharomyces cerevisiae*

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ABSTRACT

The *RAD3* gene of *Saccharomyces cerevisiae* is required for excision repair and is essential for cell viability. *RAD3* encoded protein possesses a single stranded DNA-dependent ATPase and DNA and DNA·RNA helicase activities. Mutational studies have indicated a requirement for the RAD3 helicase activities in excision repair. To examine the extent of conservation of structure and function of RAD3 during eukaryotic evolution, we have cloned the *RAD3* homolog, *rhp3*$^+$, from the distantly related yeast *Schizosaccharomyces pombe*. *RAD3* and *rhp3*$^+$ encoded proteins are highly similar, sharing 67% identical amino acids. We show that like *RAD3*, *rhp3*$^+$ is indispensable for excision repair and cell viability, and our studies indicate a requirement of the putative rhp3$^+$ DNA helicase activity in DNA repair. We find that the *RAD3* and *rhp3*$^+$ genes can functionally substitute for one another. The level of complementation provided by the *rhp3*$^+$ gene in *S.cerevisiae rad3* mutants or by the *RAD3* gene in *S.pombe rhp3* mutants is remarkable in that both the excision repair and viability defects in both yeasts are restored to wild type levels. These observations suggest a parallel evolutionary conservation of other protein components with which RAD3 interacts in mediating its DNA repair and viability functions.

INTRODUCTION

Excision repair of ultraviolet light damaged DNA in eukaryotes is a complex process involving a large number of genes. In the yeast *Saccharomyces cerevisiae*, six genes, *RAD1, RAD2, RAD3, RAD4, RAD10,* and *RAD14*, are known to be required for the incision step in excision repair of UV damaged DNA (1–3), whereas several others, *RAD7, RAD16, RAD23,* and *MMS19*, affect the proficiency of excision repair (2,4,5). In humans, seven xeroderma pigmentosum (XP) complementation groups, *XPA* through *XPG*, have been identified (6,7). XP cells are defective in the incision of UV damaged DNA and as a consequence, XP patients are highly sensitive to sun light and suffer from a high incidence of skin cancers. Eight complementation groups have been identified among UV sensitive rodent cell lines and mutants from five of these groups are defective in incision (8,9). Three human excision repair genes, *ERCC1, ERCC2,* and *ERCC3,* have been cloned by complementing the UV sensitivity of rodent cell lines, and all three genes show homology to *S. cerevisiae* genes. *ERCC1* is homologous to *RAD10* (10), and *ERCC2* is a homolog of *RAD3* (11). The *ERCC3* gene complements the excision repair defect in *XP-B* mutant cells, and a homolog of this gene has been identified in *S. cerevisiae* (12). Recently, we have found that the *S. cerevisiae RAD14* gene is a homolog of the human *XPAC* gene (3). The conservation of excision repair genes between yeast and human implies that information gleaned from the yeast system would be applicable to higher eukaryotes, including humans.

The *S. cerevisiae RAD3* gene is of particular interest because in addition to its requirement in excision repair, it is essential for cell viability (13,14). The *RAD3* encoded protein possesses single stranded DNA-dependent ATPase (15) and DNA helicase activities, and RAD3 helicase translocates 5′ → 3′ on single stranded DNA (16). RAD3 also unwinds DNA·RNA duplexes and this activity is similar in efficiency to the DNA helicase activity (17). Our studies with the *rad3 Arg-48* mutation, in which the lysine 48 residue in RAD3 has been changed to arginine, have indicated a requirement for RAD3 helicase activities in excision repair. The rad3 Arg-48 mutant protein is defective in the ATPase and helicase activities, but it can still bind ATP. The *rad3 Arg-48* mutation confers a reduced level of incision of UV damaged DNA and also a defect in a post incision step of excision repair (18). The RAD3 DNA and DNA·RNA helicase activities could be involved in tracking of DNA for damaged sites (17); in addition, the RAD3 DNA helicase could function in the turnover of the incision complex from the damage site, or of the oligonucleotide fragment following the incision reaction, or both (18).

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To gain insight into the extent of conservation in the structure and function of RAD3, we have characterized the RAD3 homolog, *rhp3*$^+$ (rad homolog of *S. pombe*-3) from the evolutionarily divergent fission yeast *Schizosaccharomyces pombe*. *S. pombe* resembles higher eukaryotes more closely than does *S. cerevisiae*, as indicated from phylogenetic studies of 5 S ribosomal RNAs (19), control of the mitotic cell cycle, presence of introns in many *S. pombe* genes, and sequence requirements for splicing of introns (20,21). We show that *rhp3*$^+$ is required for both DNA repair and cell viability, and that *RAD3* and *rhp3*$^+$ can fully substitute for one another in both functions.

MATERIALS AND METHODS

Yeast strains, plasmids, and genetic methods

A list of yeast strains and plasmids used is given in Table 1. Standard genetic techniques and growth media for *S. cerevisiae* were used (22). Genetic techniques and media for *S. pombe* were

Table 1. Strains and plasmids

A. Yeast strains

Strain		Genotype
Saccharomyces cerevisiae		
YR3-3	MATα	leu2-3,112 ura3-52 can1 rad3-2 trp1Δ::ura3 (by FOA)
LP3041-6D	MATa	leu2-3,112 ura3-52 trp1Δ
YR3-15		YR3-3×LP3041-6D, (rad3-2/rad3Δ::LEU2)
PRY274		YR3-15 + pRR567 (ADC1:: rhp3$^+$)
(PRY277-280 originate from one tetrad after sporulating PRY274)		
PRY277		leu2-3,112 ura3-52 trp1Δ rad3Δ::LEU2 + pRR567
PRY278		leu2-3,112 ura3-52 trp1Δ rad3-2
PRY279		leu2-3,112 ura3-52 trp1Δ rad3-2 +pRR567
PRY280		leu2-3,112 ura3-52 trp1Δ rad3Δ::LEU2 +pRR567
DBY747arg-48	MATa	*his3*Δ1 leu2-3,112 trp1-289 ura3-52 rad3 Arg-48
PRY290		DBY747 rad3 Arg-48 +pRR567
Schizosaccharomyces pombe		
Haploid		
PRZ107		h$^+$ leu1-32 ura4-D18 lys1-131 ade6-210 rhp3$^+$
PRZ110		h$^-$ leu1-32 ura4-D18 lys1-131 ade6-216 rhp3$^+$
PRZ155		h$^-$ leu1-32 ura4-D18 ade6-216 rad15
PRZ160		PRZ155 + pRR402
PRZ161		PRZ155 + pRR541
PRZ162		PRZ155 + pRR543
PRZ163		PRZ155 + pRR551
PRZ176		PRZ110 + pRR541
PRZ185		PRZ155 + pRR586
PRZ192		PRZ110 rhp3Δ::LEU2 + pRR541
PRZ193		PRZ110 rhp3Δ::LEU2 + pRR543
PRZ195		PRZ107 rhp3Δ::LEU2 + pRR586
Diploid		
ZD19		PRZ107×PRZ110 (rhp3$^+$/rhp3Δ::LEU2)
ZD22		PRZ107×PRZ110 (rhp3$^+$/rhp3$^+$)
ZD40		ZD19 + pRR402
ZD41		ZD19 + pRR541
ZD42		ZD19 + pRR543
ZD43		ZD19 + pRR551

B. Plasmids

Plasmid	Description
pRR387	pURA4 derivative with single EcoRI site at former BamHI site
pRR402	pURA4 derivative with S. pombe ADH promoter upstream of multiple cloning site (MCS). Promoter is from pEVP11.
pRR535	pURA4 derivative with S. pombe rhp6$^+$ promoter upstream of MCS
pRR541	pRR402 with RAD3 ORF downstream of S. pombe ADH promoter
pRR543	pRR535 with RAD3 ORF downstream of S. pombe rhp6$^+$ promoter
pRR551	pRR387 with 4.4 kb EcoRI (ClaI)-EcoRI fragment containing rhp3$^+$
pRR586	pRR387 with 4.4 kb EcoRI (ClaI)-EcoRI fragment containing rhp3 Arg-48
pSCW231	S. cerevisiae ADC1 vector
pRR567	pSCW231 with rhp3$^+$ ORF downstream of S. cerevisiae ADC1 promoter
pRR508	pUC18 derivative with the 7.5 kb SalI-BamHI DNA fragment (Fig. 1a) containing the entire rhp3$^+$ gene in which the BglII (+483) to XbaI (+1668) fragment has been replaced by the S. cerevisiae LEU2$^+$ gene. This plasmid is used for generating a genomic rhp3Δ strain of S. pombe by digestion with PstI and BamHI and transformation to Leu2$^+$

as described (23,24). Yeast transformations were performed according to Ito et al. (25), and *E. coli* transformations were carried out according to Maniatis et al. (26). Recovery of plasmid DNA from yeast strains was as previously described (27).

Cloning and sequence analysis of the *rhp3*$^+$ gene

The *RAD3* DNA used as a probe for identifying homologous sequences in *S. pombe* by Southern hybridization was a 2.4 kb *Kpn*I-*Eco*RI fragment corresponding to positions −479 to +1918 in *RAD3* (28). For cloning the *rhp3*$^+$ gene, an *S. pombe* genomic library constructed in the λ vector EMBL3 (29) was used. Initial restriction analysis of DNA prepared from 10 positive clones according to Davis et al. (30) indicated that they all overlapped. A 7.5 kb *Sal*I-*Bam*HI fragment within one of the clones was identified by Southern blotting as containing the *S. pombe* sequence that hybridized to *RAD3* (Fig. 1). The nucleotide sequence of the *rhp3*$^+$ gene was determined by the dideoxy chain termination method (31) using ([α-^{35}S]thio)triphosphate (32).

Generation of a genomic *rhp3*Δ mutation in *S. pombe*

The *Bgl*II-*Xba*I fragment in the *rhp3*$^+$ open reading frame (ORF) from position +483 to +1668 was deleted and replaced with a 1.9 kb *Bam*HI-*Xba*I fragment containing the *S. cerevisiae LEU2* gene, generating plasmid pRR508. This replacement deletes a little over half of the rhp3 ORF.

We made the *rhp3*Δ mutation in a wild-type *S. pombe* haploid strain harboring the *RAD3* gene on plasmid pRR541 (*S. pombe ADH* promoter::*RAD3* gene) or pRR543 (*S. pombe rhp6*$^+$ promoter::*RAD3* gene). These haploids were transformed with the linear *Pst*I-*Bam*HI fragment from pRR508 in which the *rhp3*$^+$ gene from *Bgl*II to *Xba*I within the rhp3 ORF is replaced with the *LEU2* gene. Leu$^+$ transformants were screened for inability to grow on 5-FOA, indicating the requirement for the *RAD3 URA3* plasmid. The generation of the *rhp3*Δ mutation was confirmed by Southern blot analysis.

Site-directed mutagenesis of the lysine-48 codon to arginine within the *rhp3*$^+$ gene

The lysine 48 codon (AAG) was changed to the arginine codon AGG by the method of Kunkel et al. (33), using, for mutagenesis, the oligonucleotide 5′-GAAATGGTCCTTCCAGTAC-3′, which is complementary to the mRNA equivalent DNA strand from position +134 to +152 in the *rhp3*$^+$ ORF. The identity of the mutation was verified by the presence of a new *Sau*96I restriction site. Plasmid pRR586 carries the *rhp3 Arg-48* mutant gene on a multicopy *S. pombe* vector.

Plasmids for expression of *rhp3*$^+$ in *S. cerevisiae* and of *RAD3* in *S. pombe*

Plasmid pRR567 carries the *rhp3*$^+$ gene fused downstream of the *S. cerevisiae ADC1* promoter in the multicopy vector pSCW231. Two *S. pombe* vectors, both derivatives of pURA4 (34), a multicopy ars-based plasmid, were constructed for expression of the *S. cerevisiae RAD3* gene in *S. pombe*, and *RAD3* was fused to the *S. pombe ADH* promoter (35) as well as to the *S. pombe rhp6*$^+$ promoter (36). Plasmid pRR541 contains the *S. pombe ADH* promoter::*S. cerevisiae RAD3* gene fusion. The *RAD3* gene was cloned downstream of the *rhp6*$^+$ promoter in plasmid pRR543.

RESULTS

Sequence of the *rhp3*$^+$ gene and homology to *S. cerevisiae RAD3* and human *ERCC2* genes

The sequence of the three contiguous *Hin*dIII fragments carrying the *rhp3*$^+$ gene (Fig. 1) was determined and has been deposited in the EMBL databank (Accession no. X64583). A single ORF is present in this sequence, and it encodes a protein of 772 amino acids with a predicted M_r of 88, 175. The *rhp3*$^+$ gene does not contain any introns. Fig. 2 shows the alignment of protein sequences encoded by the *S. cerevisiae RAD3*, *S. pombe rhp3*$^+$ and human *ERCC2* genes. The *RAD3*, *rhp3*$^+$ and *ERCC2* genes encode nearly similar length proteins of 778, 772, and 760 amino acids, respectively. The three proteins share a high degree of homology throughout their length and the various consensus motifs identified among DNA helicases have been highly conserved among them (38). A total of 11 sites have been identified in *RAD3* where missense mutations result in a UV sensitive phenotype and mutation of one site is associated with a temperature sensitive for growth phenotype (39). All these amino acids are identical in the three proteins. rhp3$^+$ protein shares ~67% and 56% identical residues with RAD3 and ERCC2 proteins, respectively, and RAD3 and ERRC2 proteins share 53% identical residues. Grouping of conservative amino acid substitutions with identical residues raises the homology to ~82% between rhp3$^+$ and RAD3 and to 73% to 75% among the two yeast and human proteins.

rhp3$^+$ is required for cell viability and DNA repair

To determine if *rhp3*$^+$ is essential for cell viability, we constructed a genomic deletion mutation of this gene. An internal section of the *rhp3*$^+$ ORF, from *Bgl*II at position +483 to *Xba*I at +1668 (Fig. 1), was deleted and replaced with the *S. cerevisiae*

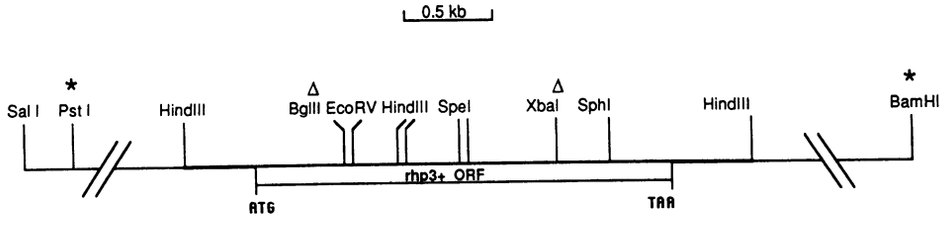

Figure 1. Restriction map of the *rhp3*$^+$ gene and flanking regions. Partial restriction map of the 7.5 kb *Sal*I-*Bam*HI fragment isolated from the bacteriophage λ vector EMBL-3 harboring the *S. pombe* genomic sequence hybridizing to the *S. cerevisiae RAD3* probe. The *rhp3*$^+$ gene region is indicated by a bold line and the size scale applies to this region only. The *Pst*I and *Bam*HI sites used to construct the linear DNA fragment for generating a genomic *rhp3*Δ mutation are denoted by an asterisk (*). In the genomic *rhp3*Δ mutations, the *rhp3*$^+$ sequence was deleted between the *Bgl*II and *Xba*I sites marked with a triangle (Δ) and replaced by the *S. cerevisiae LEU2* gene.

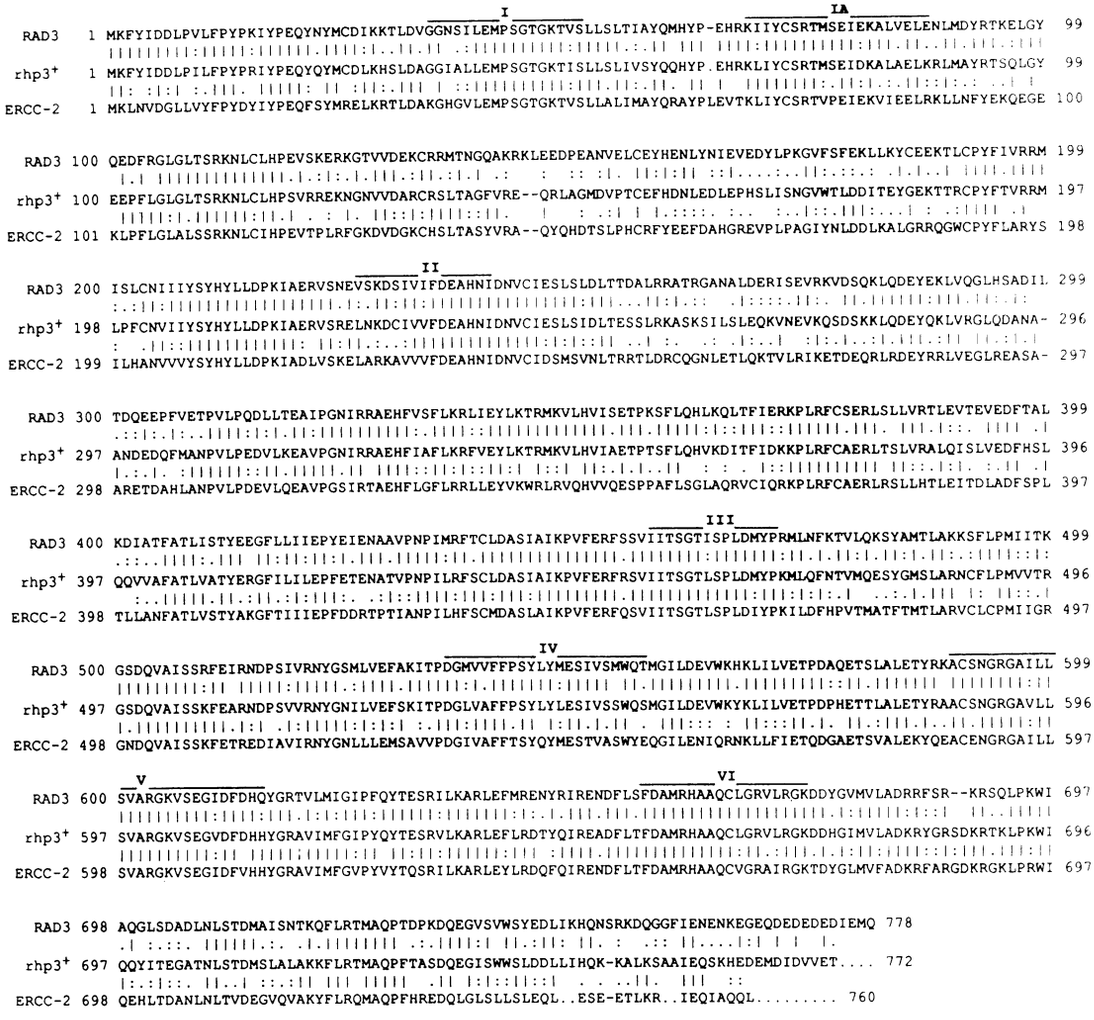

Figure 2. Homology between the *S. pombe rhp3*$^+$, *S. cerevisiae RAD3*, and human *ERCC-2* encoded proteins. The sequences were aligned using the GAP program of the Genetics Computer Group, Inc. (37). Symbols: |, identical residues; :, highly conserved residues; ·, weakly conserved residues. Horizontal lines with Roman numerals above the sequences denote the consensus sequences of the helicase superfamily as described by Gorbalenya et al. (38). References for sequences are: *RAD3* (28), *ERCC2 (11)*, rhp3$^+$ (EMBL accession no. X64583).

LEU2 gene in one of the homologs in a diploid *rhp3*$^+$/*rhp3*$^+$ *S. pombe* strain. The *rhp3*Δ::LEU2/rhp3$^+$ genotype of the resulting Leu$^+$ transformants was confirmed by Southern analyses. Sporulation and tetrad analyses of several such diploids produced at most 2 viable spores and none of the viable spores were Leu$^+$. The results of tetrad analysis in one such diploid, ZD19, are shown in Fig. 3a. In a total of 57 asci dissected from this strain, a majority of tetrads (~85%) gave 2 viable spores, while the remaining tetrads gave only 1 viable spore, and all viable spores were Leu$^-$.In contrast, sporulation of the parental *rhp3*$^+$/*rhp3*$^+$ diploid strain ZD22 gave 4 viable spores in most of the tetrads (Fig. 3b). Thus, like *RAD3*, *rhp3*$^+$ is an essential gene.

The *S. pombe RAD3* homolog has been independently cloned by A. Lehmann and collaborators (personal communication) by complementing the UV sensitivity of the *S. pombe rad15* mutant strain (40). As expected, the *rhp3*$^+$ gene restores the UV sensitivity of the *rad15* mutant to wild type levels (Fig. 4). The rad3 Arg-48 mutant protein, in which the lysine 48 residue has been changed to arginine, is defective in single stranded DNA-dependent ATPase and DNA helicase activities (18). This mutation does not affect the cell viability function of *RAD3*, but confers a defect in excision repair. To examine the cellular role of the putative rhp3$^+$ DNA helicase activity, we changed the lysine 48 residue in the *rhp3*$^+$ gene to arginine. An *rhp3*$^+$ haploid strain was transformed with plasmid pRR586 carrying the *rhp3 Arg-48* mutant gene. The genomic *rhp3*$^+$ gene was then deleted and replaced by the *LEU2* gene. The creation of the *rhp3*Δ::LEU2 mutation was confirmed by Southern analyses. The *rhp3*Δ strain carrying the *rhp3 Arg-48* mutant gene was viable and grew as well as the *rhp3*$^+$ wild type strain (data not shown). However, the *rhp3 Arg-48* mutation conferred UV sensitivity nearly similar to that of the *S. pombe rad15* mutant strain, and the *rhp3 Arg-48* gene did not complement the UV sensitivity of the *rad15* strain (Fig. 4). These observations indicate that, like RAD3 helicase, the putative *rhp3*$^+$ helicase activity is required for the DNA repair function but not for viability.

Complementation of viability and DNA repair defects of *rhp3* mutations by *RAD3*

Because *rhp3*$^+$ resembles *RAD3* in its requirement for cell viability and DNA repair, we next determined if the *RAD3* and *rhp3*$^+$ genes are functionally interchangeable. To examine if

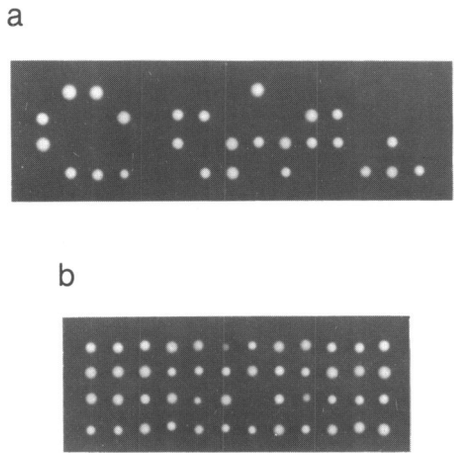

Figure 3. *rhp3*$^+$ is an essential gene. (a). Tetrad analysis of asci from the diploid *S. pombe* (*rhp3*$^+$/*rhp3*Δ) strain, ZD19. (b). Tetrad analysis of asci from the isogenic diploid *rhp3*$^+$/*rhp3*$^+$ strain ZD22.

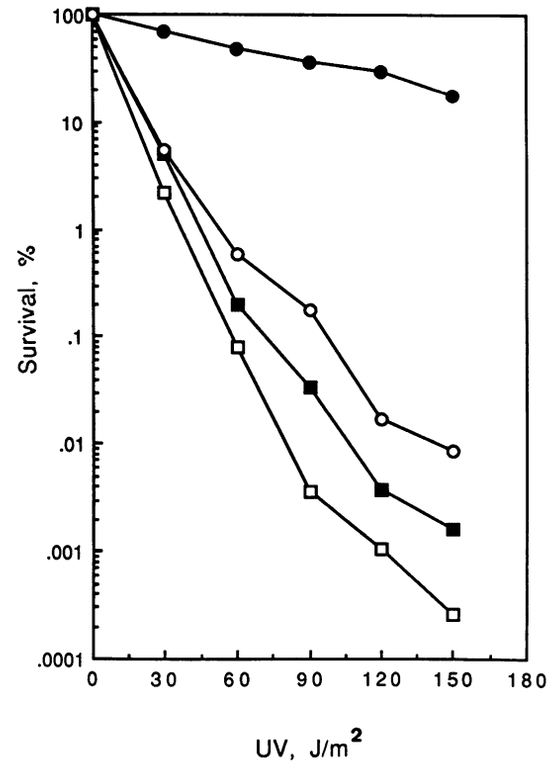

Figure 4. UV sensitivity of *S. pombe rad15* and *rhp3 arg-48* mutant strains. Symbols: □, PRZ160 (*rad15* + vector); ●, PRZ163 (*rad15* + *rhp3*$^+$ gene in plasmid pRR551), ■, PRZ185 (*rad15* + *rhp3 arg-48* gene in plasmid pRR586); ○, PRZ195 (*rhp3*Δ + *rhp3 arg-48* gene in plasmid pRR586).

RAD3 complements the viability and DNA repair defects of *rhp3* mutations in *S. pombe*, we constructed plasmids pRR541 and pRR543 to express the *RAD3* gene in *S. pombe*. In pRR541, *RAD3* is placed downstream of the *S. pombe ADH* promoter, whereas in pRR543 it is fused to the *S. pombe rhp6*$^+$ promoter (36). Western blots of total cellular protein from *S. pombe* strains carrying these plasmids were probed with anti-RAD3 antibodies. The level of RAD3 protein produced from plasmid pRR541 in *S. pombe* was approximately 10-fold higher than the wild type level of rhp3$^+$ protein in *S. pombe*. In contrast, the amount of RAD3 in *S. pombe* cells carrying pRR543 was about the same as wild type rhp3$^+$ levels in *S. pombe* (data not shown).

The *rhp3*Δ S. pombe strain carrying the *RAD3* gene on plasmid pRR541 or pRR543 was obtained as described in Materials and Methods. The generation of the genomic *rhp3*Δ mutation was verified by Southern blot analysis. The presence of the *RAD3* plasmid in these strains was confirmed by restriction analyses following their isolation and amplification in *E. coli*. The *RAD3* gene carried on plasmid pRR541 or pRR543 restored wild type levels of viability and growth to the *rhp3*Δ strain (Fig. 5a). *RAD3* also replaced the DNA repair function of *rhp3*$^+$ in *S. pombe*. The *rhp3*Δ strain carrying the *RAD3* gene on plasmid pRR541 or on plasmid pRR543 had wild type level of UV resistance. Introduction of these *RAD3* plasmids in the *S. pombe rad15* strain also raised the UV resistance to near wild type level (Fig. 5b).

Complementation of viability and DNA repair defects of *rad3* mutations by *rhp3*$^+$

To determine if the *rhp3*$^+$ gene can functionally substitute for the *RAD3* gene in *S. cerevisiae*, we expressed the *rhp3*$^+$ gene in *S. cerevisiae* by fusing it downstream of the *S. cerevisiae ADC1* promoter in plasmid pSCW231 (15), creating pRR567. As judged by Western analysis using anti-RAD3 antibodies, the level of rhp3$^+$ protein in *S. cerevisiae* cells carrying the plasmid pRR567 was only a few fold higher than the wild type level of RAD3 protein. The recognition of rhp3$^+$ protein by polyclonal anti-RAD3 antibodies is not unexpected, given the high degree of homology between the two proteins. Transformation of the UV sensitive *rad3-2*/*rad3*Δ::LEU2 diploid strain YR3-15 with plasmid pRR567 made it UV resistant. Strain YR3-15 harboring either the *rhp3*$^+$ plasmid pRR567 or the vector pSCW231 was sporulated and subjected to tetrad analysis. As expected, YR3-15 containing pSCW231 gave only 2 viable spores and all of these spores were Leu$^-$. In contrast, YR3-15 containing pRR567 produced many tetrads with 4 viable spores and these showed 2:2 segregation for Leu$^+$ and Leu$^-$. The Leu$^+$ spores always carried the plasmid linked Trp$^+$ marker, indicating that the *rhp3*$^+$ gene on plasmid pRR567 is able to restore viability to the *rad3*Δ spores. The 4 spores from one of these tetrads were selected for further study. The relevant phenotypes of these four strains were as follows: PRY277, UV^R Leu$^+$ Trp$^+$; PRY278, UV^S Leu$^-$ Trp$^-$; PRY279, UV^R Leu$^-$ Trp$^+$; PRY280, UV^R Leu$^+$ Trp$^+$. In addition to genetic markers, other lines of evidence were used to confirm the genetic constitution of these strains. First, the presence of the *rad3*Δ mutation, as indicated by the Leu$^+$ phenotype in strains PRY277 and PRY280, was verified by probing genomic Southern blots of these strains with the *RAD3* sequence. Second, the presence of the plasmid pRR567, as indicated by the plasmid linked Trp$^+$ phenotype in PRY277, PRY279, and PRY280, was confirmed by restriction analyses of plasmid DNA isolated from these strains. Third, Western blots of total cellular protein from PRY277, PRY278, PRY279, and PRY280 probed with anti-RAD3 antibody showed the rhp3$^+$ protein in the three Trp$^+$ strains but not in the Trp$^-$ strain (data not shown).

The *rhp3*$^+$ gene conferred wild type levels of growth to the *rad3*Δ strain (Fig. 6a). *rhp3*$^+$ also restored wild type level of UV resistance in the *rad3-2*, *rad3 Arg-48*, and *rad3*Δ strains

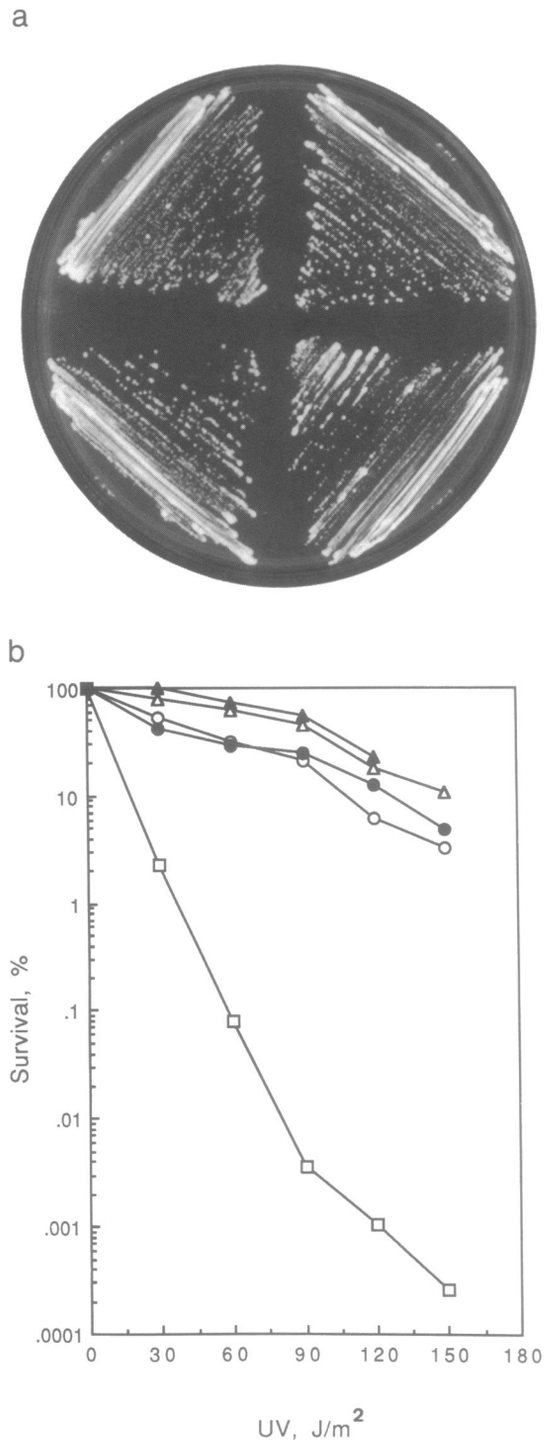

Figure 5. Complementation of viability and DNA repair defects of *S. pombe rhp3Δ* and *rad15* mutations by the *S. cerevisiae RAD3* gene. (a). *RAD3* confers wild type levels of growth to the *rhp3Δ* mutant. Upper left, PRZ110 *(rhp3$^+$)*; upper right, PRZ193 *(rhp3Δ + rhp6$^+$ promoter::RAD3* gene in plasmid pRR543); lower left, PRZ176 *(rhp3$^+$ + ADH promoter::RAD3* gene in plasmid pRR541); lower right, PRZ192 *(rhp3Δ* + ADH promoter::RAD3 gene in plasmid pRR541). Cells from a single colony were streaked onto YE medium and incubated at 30°C for 3 days. (b). *RAD3* restores wild type levels of UV resistance in *S. pombe rad15* and *rhp3Δ* mutants. Symbols, □, PRZ160 *(rad15* + vector); ○, PRZ161 *(rad15 + ADH promoter::RAD3* gene in plasmid pRR541); ●, PRZ162 *(rad15 + rhp6$^+$ promoter::RAD3* gene in plasmid pRR543); ▲, PRZ192 *(rhp3Δ* + ADH promoter::RAD3 gene in plasmid pRR541); △, PRZ193 *(rhp3Δ* + rhp6$^+$ promoter::RAD3 gene in plasmid pRR543).

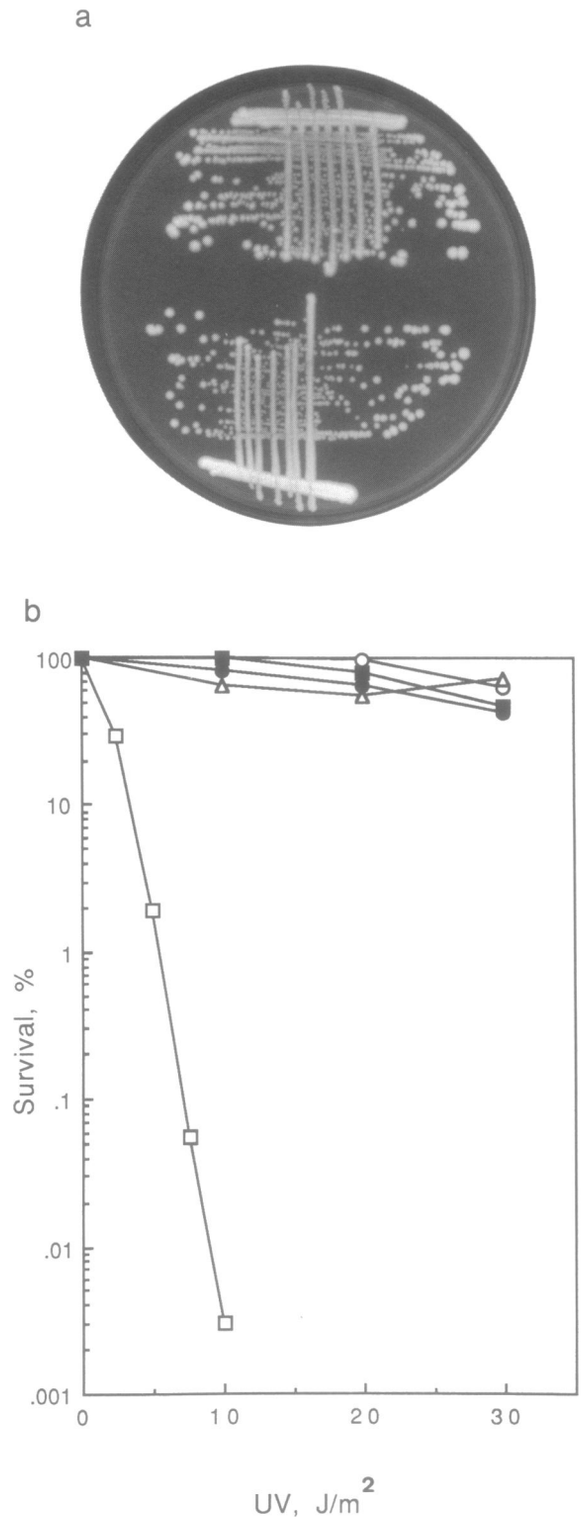

Figure 6. *rhp3$^+$* restores viability and DNA repair in *rad3* mutants to wild type levels. (a). The *rhp3$^+$* gene confers normal growth in a *rad3Δ* strain. Top: LP3041−6D *(RAD3$^+$)*; Bottom, PRY277 *(rad3Δ + rhp3$^+$* gene in plasmid pRR567). Cells from a single colony were streaked onto YPD medium and incubated at 30°C for 3 days. (b) *rhp3$^+$* complements the DNA repair defect of *rad3* mutants. Symbols: ○, PRY277 *(rad3Δ + rhp3$^+$* gene in plasmid pRR567); □, PRY278 *(rad3-2)*; ■, PRY279 *(rad3-2* + pRR567); △, PRY280 *(rad3Δ* + pRR567); ●, PRY290 *(rad3 Arg48* strain + pRR567). The UV sensitivity of the *S. cerevisiae rad3 Arg48* mutant is identical to that of the *rad3-2* strain.

(Fig. 6b). The complementation of UV sensitivity of *rad3* by the *rhp3*$^+$ gene is specific, as we found no evidence of increased UV resistance when the *rhp3*$^+$ gene on plasmid pRR567 was introduced into other *S. cerevisiae* excision defective mutants, *rad1*Δ, rad2Δ, rad4Δ, and *rad10*Δ.

DISCUSSION

In this paper, we show that the *rhp3*$^+$ gene of *S. pombe* is a homolog of the *S. cerevisiae RAD3* gene and of the human *ERCC2* gene. The three proteins share extensive amino acid similarity throughout their length, and the homology is particularly strong in the seven conserved helicase domains. The major difference among the proteins is in the carboxyl terminus, where RAD3 contains 13 carboxyl terminal residues, 9 of which are acidic. These 13 residues are missing in the ERCC2 protein and the rhp3$^+$ protein lacks 4 of the carboxyl terminal residues present in RAD3. The carboxyl terminus of RAD3, however, is not essential for function, as its deletion produces no adverse effect on the excision repair or the viability function of *RAD3* (28).

Our genetic studies with the *rhp3*$^+$ gene establish that like *RAD3, rhp3*$^+$ is required for DNA repair and cell viability. The *rhp3*$^+$ gene complements the UV sensitivity of the *S. pombe rad15* mutation; however, since this mutation, like the other *S. pombe rad* mutations is not well characterized, the involvement of *rhp3*$^+$ *(rad15)* in excision repair or in cell viability was previously unknown. To examine the role of the putative DNA helicase activity in *rhp3*$^+$, we mutated the lysine 48 residue to arginine in the conserved nucleotide binding sequence present in the rhp3$^+$ protein. Based on our studies with the rad3 Arg-48 protein, we expect the rhp3 Arg-48 protein to be defective in single strand DNA-dependent ATPase and in DNA and DNA·RNA helicase activities. The *rhp3 Arg-48* mutation inactivated DNA repair but had no effect on cell viability, indicating a requirement of rhp3$^+$ helicase activities in DNA repair.

The most remarkable findings in this work relate to the very high degree with which the *RAD3* and *rhp3*$^+$ genes can functionally substitute for one another. The *S. cerevisiae RAD3* gene complemented the viability defect of the *S. pombe rhp3*Δ mutant strain, and the *rhp3*Δ strain carrying the *RAD3*-bearing plasmid grew at wild type rate. *RAD3* also complemented the UV sensitivity of the *S. pombe rad15* and *rhp3*Δ mutant strains to wild type levels. The wild type level of complementation by *RAD3* was achieved even when the amount of RAD3 protein in *S. pombe* cells was about the same as the level of the wild type rhp3$^+$ protein in *S. pombe*. The reciprocal experiments showed that *rhp3*$^+$ complements the DNA repair and viability defects of various *rad3* mutants to wild type levels. The *rhp3*$^+$ gene provided normal growth rate to the *rad3*Δ strain and increased the UV resistance of the *rad3-2, rad3 Arg-48* and *rad3*Δ mutant strains to wild type levels. The *rad3-2* mutation confers a total defect in incision of UV damaged DNA (2), whereas the *rad3 Arg-48* mutation causes a reduction in the level of incision of UV damaged DNA as well as a defect in the post-incision step of excision repair (18). The fact that *rhp3*$^+$ restores normal UV resistance in these *rad3* mutant strains establishes a role of the *rhp3*$^+$ gene in the incision process as well as in the post-incision step of excision repair.

The DNA repair and viability roles of RAD3 are mutationally separable, suggesting the possibility that RAD3 interacts with different protein components in mediating these functions. Even though RAD3 helicase activity, as well as the putative rhp3$^+$ helicase activity, is not essential for viability, the RAD3 function may still be required in some aspects of DNA replication (18). The very high degree of structural and functional conservation of RAD3 implies a high level of conservation of other proteins with which RAD3 interacts in mediating its role in excision repair and in the maintenance of cell viability.

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