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Saccharomyces cerevisiae exonuclease-1 plays a role in UV resistance that is distinct from nucleotide excision repair

Junzhuan Qiu, Min-Xin Guan[2], Adam M. Bailis[1] and Binghui Shen*

Department of Cell and Tumor Biology and [1]Department of Molecular Biology, The Beckman Research Institute of City of Hope, Duarte, CA 91010-3000, USA and [2]Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA

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ABSTRACT

Two closely related genes, *EXO1* and *DIN7*, in the budding yeast *Saccharomyces cerevisiae* have been found to be sequence homologs of the *exo1* gene from the fission yeast *Schizosaccharomyces pombe*. The proteins encoded by these genes belong to the Rad2/XPG and Rad27/FEN-1 families, which are structure-specific nucleases functioning in DNA repair. An XPG nuclease deficiency in humans is one cause of xeroderma pigmentosum and those afflicted display a hypersensitivity to UV light. Deletion of the *RAD2* gene in *S.cerevisiae* also causes UV hypersensitivity, due to a defect in nucleotide excision repair (NER), but residual UV resistance remains. In this report, we describe evidence for the residual repair of UV damage to DNA that is dependent upon Exo1 nuclease. Expression of the *EXO1* gene is UV inducible. Genetic analysis indicates that the *EXO1* gene is involved in a NER-independent pathway for UV repair, as *exo1 rad2* double mutants are more sensitive to UV than either the *rad2* or *exo1* single mutants. Since the roles of *EXO1* in mismatch repair and recombination have been established, double mutants were constructed to examine the possible relationship between the role of *EXO1* in UV resistance and its roles in other pathways for repair of UV damaged DNA. The *exo1 msh2*, *exo1 rad51*, *rad2 rad51* and *rad2 msh2* double mutants were all more sensitive to UV than their respective pairs of single mutants. This suggests that the observed UV sensitivity of the *exo1* deletion mutant is unlikely to be due to its functional deficiencies in MMR, recombination or NER. Further, it suggests that the *EXO1*, *RAD51* and *MSH2* genes control independent mechanisms for the maintenance of UV resistance.

INTRODUCTION

Multiple DNA repair pathways exist to counteract the deleterious effects of UV irradiation on cellular DNA. One of the widely distributed repair enzymes, photolyase, which utilizes visible light to reverse UV-induced DNA damage, is conserved from prokaryotic to eukaryotic cells (1). Another important and conserved pathway for the repair of UV damage to DNA is nucleotide excision repair (NER). This pathway works by incising DNA on either side of the damage site with endonucleases, removing a 29 bp oligonucleotide with a helicase and then resealing the gap with polymerase and ligase. Seventeen polypeptides are required in this process in mammalian cells (2). Defects in the polypeptides that execute the early stages of recognition and cleavage cause human genetic diseases, including xeroderma pigmentosum, which predisposes patients to skin cancer (2–4).

NER is a mechanism by which cells can excise UV photoproducts, such as cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts (6–4 PPs) from DNA. In *Saccharomyces cerevisiae*, mutation of the genes that encode key NER enzymes causes dramatic UV sensitivity (5–7). However, in the fission yeast *Schizosaccharomyces pombe* deletion of the NER genes confers less sensitivity to UV, such that the mutants retain a substantial ability to remove UV photoproducts (8,9). This implies the existence of a secondary pathway for UV damage repair in *S.pombe* and an enzyme (SPDE) that initiates this process has been discovered and preliminarily characterized (10,11).

Recently, another nuclease, exonuclease-1 (*Sp*Exo1) has been isolated from *S.pombe* (12,13). The enzyme activity and mRNA expression levels are significantly induced during meiosis, indicative of its function in mismatch correction during meiotic recombination. Exo1 is a member of a family of DNA repair nucleases that includes Rad13 and Rad2 from *S.pombe* and their human homologs XPG and FEN-1 (13). Rad13 is a key component of the NER machinery in *S.pombe*, acting as a 3′ nuclease during pyrimidine dimer removal. Accordingly, *rad13* null mutants are sensitive to UV irradiation (14). The null mutant of *rad2* is modestly sensitive to UV, implying that it plays a minor role in UV repair (15).

The *RAD2* and *RAD27* genes from *S.cerevisiae* are homologs of the *S.pombe Rad13* and *Rad2* genes respectively. Deletion of both the *RAD2* and *RAD27* genes has a synergistic effect on repair of UV-induced DNA damage in *S.cerevisiae* (16). This result indicates that there is more than one pathway for UV resistance in *S.cerevisiae* as well. Moreover, residual UV survival exists even in the *rad2 rad27* double mutant, implying the existence of

*To whom correspondence should be addressed. Tel: +1 626 301 8879; Fax: +1 626 301 8972; Email: bshen@smtplink.coh.org

Table 1. Primers used to construct and confirm mutants

Primers	Primer sequences (5' - 3')	Applications
exolpl	AGAAAGCCATGGGTATCCAAGGT	Clone *EXO1* gene into
exolp2*	CTCCGAAAGCTTTCAGTGATGATGGTGG-TGGTGTTTACCTTTATAAACAAATTGGGAA	pET28b vector
exolp3	CGAACAAACTGAAAGGCGTAG	Confirm deletion of
exolp4	GTCTTGAGGCATTTCGACGAG	*EXO1*
din7pl	CGATAGCCATGGGAATACCTGGCT	Clone *DIN7* gene into
din7p2*	CAATGGGATCCCTAATGGTGATGGTGATG-GTGGAAAATTGATGGTACGGTGCCTGA	pET28b vector
KNSCB1	CAATTAAAGAGAATTCAAAAACAGGTGTCCCTGAAA-AAATACATGTATCAAACACGTACGCTGCAGGTCGAC	Disrupt *DIN7* gene
KNSCB2	CTCCCTCTCCGATAACACGTCCTGCGTATCCACTAG-CGGTTGCTCCACTTTCTTATCGATGAATTCGAGCTCG	
din7p3	AGCGAGTAAGAGAAAACGATC	Confirm deletion of
din7p4	CGGTGGAATAAGAATACAAC	*DIN7*
TH-MSHF2	AATGTTGACACTCTACTCCA	Confirm deletion of
TH-MSHR	TTCTCACTGCAGATGTCGTTG	*MSH2*

* The long primer includes a DNA sequence encoding six histidines (Histag), which can be used to purify the enzyme after it is overexpressed. Underlined sequences were designed for the amplification of *HIS3* mark, the remaining sequences of KNSCB1 and KNSCB2 are from *DIN7*.

Table 2. *Saccharomyces cerevisiae* strains used in the study

Strain[a]	Genes deleted	Genotype	Source
W1021-7c	–	*MATa, ade2-1, can1-100, his3-11, 15, leu2-3, 112, ura3-1*	J. McDonald
W1089-6c	–	*MATα, ade2-1, can1-100, his3-11, 15, trp1-1, ura3-1*	”
FDAB13A	–	*MATα, ade2-1, can1-100, his3-11, 15, leu2-3, 112, trp1-1, ura3-1*	This study
FDAB15C	*din7*	*MATα, din7::HIS3*	”
FDAB15D	*exo1*	*MATa, exo1::URA3*	”
FDABMU	*msh2*	*MATα, msh2::hisG-URA3-hisG*	”
FDABMT	*msh2*	*MATα, msh2::TRP1*	”
135-2B	*rad2*	*MATa, rad2::TRP1*	A. Bailis
135-2A	*rad2*	*MATα, rad2::TRP1*	”
U687	*rad51*	*MATa, rad51::LEU2*	”
FDAB15A	*exo1 din7*	*MATa, exo1 ::URA3, din7::HIS3*	This study
FDEM	*exo1 msh2*	*MATα, exo1::URA3, msh2::TRP1*	”
FDER	*exo1 rad51*	*MATa, exo1::URA3, rad51::LEU2*	”
FDAR2-3B	*rad2 exo1*	*MATa, exo1::URA3, rad2::TRP1*	”
FDUR	*rad2 rad51*	*MATα, rad2::TRP1, rad51::LEU2*	”
FDRM	*rad2 msh2*	*MATa, rad2::TRP1, msh2::hisG-URA3-hisG*	”

* All the strains are derivatives of W1021-7c and W1089-6c. Differences in the genotype from the parental strains are indicated in the table.

additional pathways for UV resistance, such as recombinational repair and DNA replication bypass. Therefore, we propose that an additional Rad2-like nuclease may play a role in one of these pathways for UV resistance. One candidate is an exonuclease purified from *S.cerevisiae* that is homologous to *S.pombe* Exo1, which has been named *Sc*Exo1 (17). In order to study the role of *S.cerevisiae* homologs of *S.pombe Exo1* in DNA repair, we have cloned two genes from *S.cerevisiae* that are substantially homologous to *S.pombe Exo1*. Here we report the genetic consequences of deleting these two genes in *S.cerevisiae* and their functional relationship to NER and other UV resistance mechanisms.

MATERIALS AND METHODS

Materials

Oligonucleotide primers synthesized in the City of Hope Cancer Center core facility and used for amplifying genes and constructing null mutant strains are listed in Table 1. The yeast strains used in this study are listed in Table 2. The vector pET-28b was from Novagen (Madison, WI) and *Escherichia coli* strain XL2 blue and pBSK vector were from Stratagene (La Jolla, CA). Restriction enzymes were obtained from New England Biolabs (Beverly, MA). [α-^{32}P]dCTP was purchased from NEN (Boston, MA). Yeast culture media, including YPD, synthetic complete (SC), minimal sporulation and synthetic dextrose minimal (SD), were prepared according to Sherman *et al*. (18). Amino acids and all other medium components and chemicals were purchased from Sigma (St Louis, MO).

Sequence alignment

Sequences of the relevant genes were obtained from the NCBI protein database. They were then compared using Optimal Global Alignment of Two Sequences at EERIE (Nimes, France). The percentages of identical and similar amino acids were calculated.

Northern blotting analysis

A wild-type yeast strain W1021-7c was grown in 50 ml YPD to late log phase. Cells were harvested and resuspended in 10 ml water. The resuspended cells were spread on two sets of YPD agar

plates. One set of the plates was irradiated with 80 J/m^2 while the other set was kept as a control. Following irradiation, cells were collected from the plates, 2 ml aliquots made into four cultures, spun down, resuspended in 10 ml YPD liquid and cultured at 30°C. Cells were harvested at the indicated times after irradiation and their total RNA was isolated using RNeasy Mini Kits (Qiagen, Santa Clarita, CA). An aliquot of 10 µg total RNA was fractionated by electrophoresis in a 1.4% agarose–formaldehyde gel, transferred to a Zeta-probe membrane (BioRad) and hybridized with *EXO1* and *ACT1*, [^{32}P]dCTP-labeled by random priming (19). The images were produced by exposing the filters to X-ray film (Kodak). Specific *EXO1* mRNA bands in Figure 2A were scanned and quantitated using the IPLabGel system (Stratagene) running MacBAS software.

Gene disruption

For construction of the *exo1* null mutant strain, the *EXO1* gene was amplified by PCR using primers with *Hin*dIII and *Nco*I sites (exo1p1 and exo1p2, Table 1) and cloned into the vector pET-28b (Novagen). The *URA*3 gene was obtained from plasmid YEp24 and inserted into two *Pst*I sites, replacing the *EXO1* coding sequence between positions 170 and 828. The fragment containing the *exo1*::*URA*3 disruption was removed from the plasmid with *Xba*I and *Not*I and transformed into yeast strains W1021-7c and W10896c (see Table 2), selecting for cells that could grow without uracil. Ura$^+$ transformants were analyzed by PCR using primers exo1p3 and exo1p4 (Table 1) and genomic Southern blotting to verify disruption of *EXO1*.

The *DIN7* gene was amplified by PCR using a pair of primers (din7p1 and din7p2, Table 1) with restriction sites *Nco*I and *Bam*HI and cloned into pBSK vector (Stratagene). The gene was disrupted by transforming with a DNA fragment containing the *HIS3* gene flanked by two 53 bp fragments homologous to nt 25–78 and 1273–1326 of the *DIN*7 coding sequence (KNSCB1 and KNSCB2 in Table 1). The *HIS*3 coding sequence was amplified from a genomic clone, HIS3MX, kindly provided by Dr K.Kuchler. His$^+$ transformants were checked for disruption of *DIN*7 using PCR (primers din7p3 and din7p4 in Table 1). Double null mutants were obtained using standard genetic techniques for manipulation of *S.cerevisiae* (18).

A *msh2* mutant was generated with strain FDAB13A using plasmid pEAI98, which contains the *hisG-URA3-hisG* universal disrupter flanked by 350 bp of DNA upstream of *MSH*2 and 1.2 kb of DNA from the 3′-end of the *MSH2* open reading frame. After digestion of pEAI 98 with *Aat*II and *Pvu*II, the *msh2*::*hisG*::*URA3*::*hisG* fragment was gel purified and transformed into FDAB13A cells. Ura$^+$ transformants were selected on minimal medium without uracil and analyzed by PCR for disruption of *MSH2*. The *msh2* single mutant transformants were crossed with a *rad2* single mutant, 135-2B, to obtain *rad2 msh2* double mutants using standard genetic techniques (18).

The *rad2 rad51* double mutant was obtained by crossing *rad2* (135-2A) and *rad51* (U687) single mutants. The *exo1 rad51* and *exo1 msh2* double mutants were obtained by crossing *exo1* (FDAB15D) with *rad51* (U687) or *msh2* (FDABFT) mutants individually. All disruption mutants established in this study are listed in Table 2.

DNA damage treatment

Strains were grown in YPD liquid to saturation at 30°C and cell density was measured with a spectrophotometer (600 nm), diluted in water and plated on YPD agar. The plates were exposed to different UV dosages (254 nm germicidal lamp) and incubated in the dark for 3–4 days at 30°C before colonies were counted. Survival rate was determined based on the ratio between colony counts with and without UV treatment. Survival curves represent the average from at least two independent experiments with two sets of isogenic strains.

To measure γ-ray survival, cells were grown to stationary phase in YPD at 30°C, washed, diluted and aliquots put into Petri dishes for irradiation. After irradiation, various dilutions were plated on YPD agar plates and incubated at 30°C for 4 days. The method of MMS treatment followed Reagan *et al*. (16), where cells are grown to stationary phase at 30°C, harvested, washed with water and resuspended in 0.1 M potassium phosphate buffer (pH 7). After treatment with MMS (0, 0.1, 0.2 and 0.3%) for 30 min at 30°C with shaking, the cells were washed four times with water, diluted and plated. The plates were incubated at 30°C for 3–4 days before counting. Calculation of survival rate for the γ-ray and MMS treatments was the same as that for UV treatment.

RESULTS

Two *S.cerevisiae* homologs of *S.pombe* Exo1

We searched the publicly available *S.cerevisiae* sequence databases (GenBank/EMBL/NCBL) using the *S.pombe* Exo1 protein sequence as a query in the BlastP search algorithm (21). This search led to the identification of two open reading frames encoding proteins with a high degree of homology to *Sp*Exo1. They are *ScEXO1* and *DIN7*. *ScEXO*1 on chromosome XI (YOR033C, accession no. Z74941) is predicted to encode a 702 amino acid protein, while *DIN7* (22) on chromosome IV (YD93208B02C, accession no. Z70202) encodes a 430 amino acid protein. Using primers designed from these sequences (exop1 and exop2 for the *EXO*1 gene and din7p1 and din7p2 for the *DIN*7 gene, Table 1), both genes were obtained by PCR and cloned into plasmid vectors pET28b and pBSK respectively and sequenced. The nucleotide sequence of the coding regions of these two genes are identical to the sequences deposited in the databases.

Comparison of the predicted polypeptide sequences for *Sc*Exo1, *Sc*Din7 and *Sp*Exo1 revealed a conserved nuclease domain at their N-termini, from amino acid residue 1 to 370. The amino acid sequence identity between *Sp*Exo1 and *Sc*Exo1 and *Sc*Din7 is ~35%, while the identity between the *Sc*Exo1 and Din7 is 56%. The interspecific sequence similarity is ~65%, while the intraspecific similarity is ~85%. The protein sequence comparison for this exonuclease subfamily is depicted in Figure 1. From these data, both of the proteins appear to be structural homologs of *S.pombe* exonuclease-1.

Expression of *EXO1* is UV inducible

Previously, it was found that expression of *DIN*7, like the NER gene *RAD*2 (23,24), is UV inducible in *S.cerevisiae* (22). In order to determine the UV inducibility of the *EXO1* gene, *EXO1* mRNA levels were assessed both prior to and following exposure to UV. As shown in Figure 2A, *EXO*1 gene expression is UV inducible. The

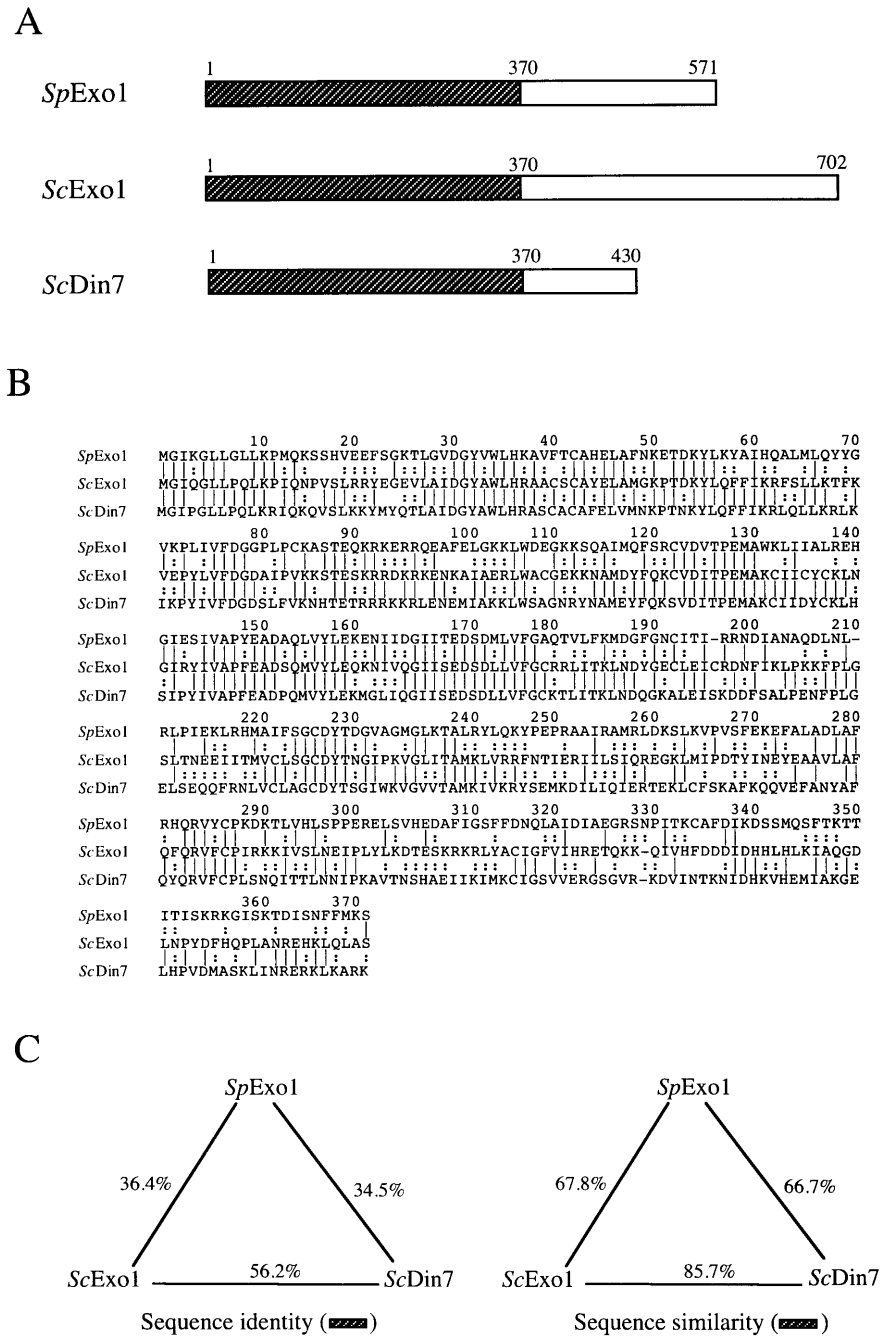

Figure 1. Sequence comparison of *S.cerevisiae* Exo1 and Din7 and *S.pombe* Exo1 proteins. (**A**) The regions of proteins compared in this study. (**B**) Alignment of the N-terminal amino acid sequences. The alignment was created using Optimal Global Alignment of Two Sequences at EERIE (Nimes, France). The identical amino acid residues are indicated by a vertical line while similar amino acids are indicated by a colon; (**C**), the percentage of identical and similar amino acid residues among N-termini of the proteins.

specific mRNA levels increased 7-fold by 5 h after UV exposure, while the *EXO1* expression levels in cells grown under the same conditions without UV treatment remained unchanged (Fig. 2B).

The role of *EXO1* and *DIN7* in DNA repair

In order to test whether *EXO1* and *DIN7* function in DNA repair, we have constructed null mutants (Table 2). Both null mutants are viable, as is the *exo1 din7* double mutant obtained from crosses. All three strains grew at the wild-type rate at both 30 and 37°C. Sensitivity to γ-ray and UV irradiation and to MMS was assessed in the single and double mutants. Neither the single mutants nor the double mutant displayed any sensitivity to γ-rays or MMS (data not shown). However, the *exo1* null mutant displayed a mild sensitivity to UV, indicating that it plays a minor role in repair of UV damage to DNA. In contrast, the *din7* mutant exhibited no sensitivity to UV, indicating that it does not play a role in UV survival. Consistent with these observations, the *exo1 din7* double mutant displayed the same UV sensitivity as the *exo1* single mutant (Fig. 3).

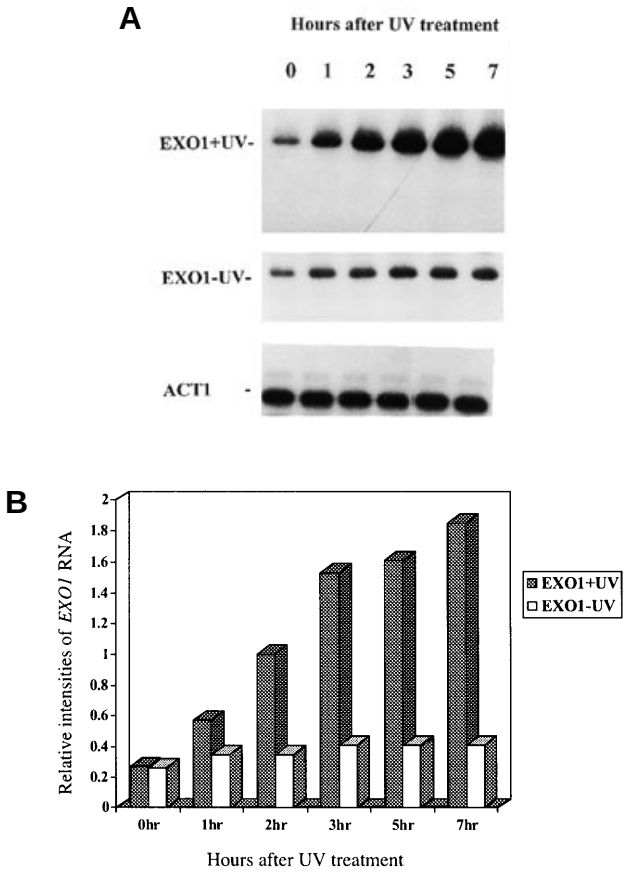

Figure 2. Gene expression in response to UV treatment. (**A**) Total RNA was prepared from the wild-type strain W1021-7c (Table 1) harvested at the time indicated after the UV treatment. For each time point, 10 µg total RNA were fractionated by electrophoresis in a 1.4% agarose–formaldehyde gel, transferred to a Zeta-probe membrane (BioRad) and hybridized with probes of *ScEXO1* encoding exonuclease-1 and *ACT1* for the yeast actin gene. Both of the DNA fragments were [^{32}P]dCTP-labeled by random priming (19). (**B**) Intensities of specific mRNA bands in (A) were scanned and quantitated using the IPLabGel system (Strategene) running MacBAS software.

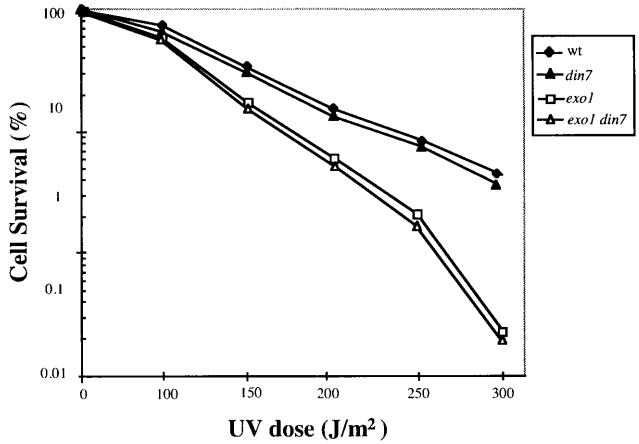

Figure 3. UV survival of wild-type, Δ*exo1*, Δ*din7* and Δ*exo1*Δ*din7* strains of *S.cerevisiae*. Isogenic strains FDAB15B, FDAB16C (WT), FDAB15D, FDAB16D (Δ*exo1*), FDAB15C, FDAB16B (Δ*din7*) and FDAB15A, FDAB16A (Δ*exo1*Δ*din7*) were grown at 30°C and cells were plated and UV irradiated, followed by incubation of the plates at 30°C in the dark. Similar results were obtained with wild-type strain W1089-6c and isogenic single mutants. The data were averaged from at least three independent experiments with two sets of isogenic strains.

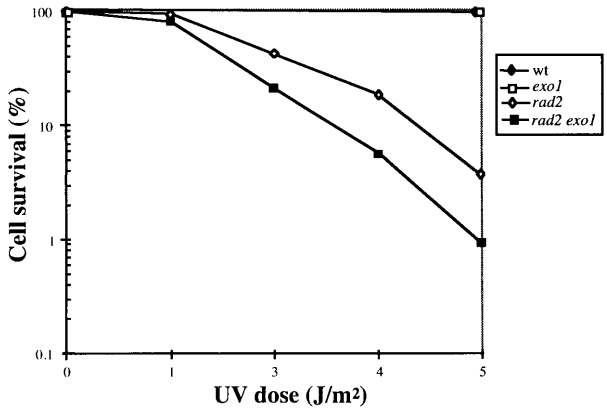

Figure 4. Epistasis analysis of the *exo1*::*URA*3 mutation. Double deletion mutant strains FDAR2-3B and FDAR2-5D were created by crossing the single mutant strains FDAB16D (Δ*exo1*) and 135-2B (Δ*rad2*). The experimental procedure is as described in Figure 3 except lower UV dosages were used. The data were averaged from at least three independent experiments with two sets of isogenic strains.

Epstasis analysis

The UV sensitivity of the *exo1* mutant suggests that *EXO1* plays a role in UV repair. As discussed previously, *EXO1* could encode a component of an alternative mechanism for UV repair that is distinct from NER. To test this, we crossed the *exo1* deletion mutant with a *rad2* null NER defective strain and analyzed UV survival of *rad2 exo1* double mutant segregants (Table 2 and Fig. 4). The results show that the double mutant is more sensitive than either the *rad2* or *exo1* single mutants, suggesting that the *EXO1* gene encodes a component of an NER-independent UV repair pathway in *S.cerevisiae*.

Because it is known that Exo1 participates in double-strand break-induced recombination (17) and also interacts with the mismatch repair protein Msh2 (25), it is possible that the UV sensitivity of the *exo1* mutant is due to a defect in double-strand break repair (DSBR) and/or mismatch repair (MMR). In order to test this possibility, we constructed double mutants of *exo1* with *msh2* and *rad51*, two major components of the MMR and DSBR pathways in *S.cerevisiae* (Table 2). When the mutants were treated with high UV dosages, the *msh2* single mutant displayed the same UV sensitivity as the *exo1* mutant, while the *rad51* deletion mutant was more UV sensitive. Interestingly, the *exo1 msh2* and *exo1 rad51* double mutants exhibited responses to UV light that were stronger than the corresponding single mutants (Fig. 5). This synergism suggests that the UV sensitivity of the *exo1* deletion mutant may not be due to deficiencies in MMR or DSBR.

To further investigate the relationship between the different DNA repair mutations with respect to UV resistance, we have extended the epistasis analysis to include the *rad2* mutation. Our results indicate that the *rad2*, *exo1*, *rad51* and *msh2* genes participate in distinct mechanisms for UV resistance because all possible double mutant combinations are more sensitive than the corresponding single mutants (Table 3).

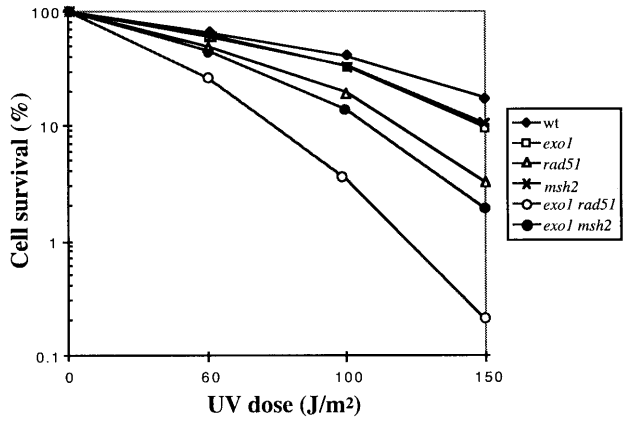

Figure 5. Synergistic effects of the *exo1* with *rad51* and *msh2* mutations. The *exo1 rad51* and *exo1 msh2* double mutants were made by crossing the individual single mutants and treating with relatively high dosages to study the synergistic effects of these double mutants. The data was averaged from at least three independent experiments with two sets of isogenic strains.

DISCUSSION

*Sc*Exo1, *Sc*Din7 and *Sp*Exo1 belong to a family of DNA repair enzymes with structure-specific nuclease activity (24,26). Based on sequence comparisons and their biochemical and biological functions in different pathways, these enzymes can be divided into three subfamilies: one includes *S.cerevisiae* Rad2 and its *S.pombe* and human homologs Rad13 and XPG; another consists of *Sc*Rad27, *Sp*Rad2 and *h*FEN-1; exonuclease-1 belongs to the third. These evolutionarily conserved enzymes may possess complementary biological functions. For example, the *Sc*Rad2 subfamily plays a major role in NER. In addition to its role in DNA replication, Rad27 also plays a minor role in UV resistance (14,16,26–29). This led us to study the role of *Sc*Exo1 and *Sc*Din7 in UV-induced DNA damage repair. Indeed, our results show that both *EXO1* and *DIN7* are UV inducible and deletion of *EXO1* confers a mild UV sensitivity similar to UVDE deletion mutants in *S.pombe* (10,30,31; see also below). Furthermore, the epistasis analysis suggests that *EXO1* does not play a role in the NER pathway, but instead is involved in a distinct mechanism of UV resistance.

Although Exo1 and Din7 are similar in their N-terminal protein sequences and UV inducibility, it is clear that *DIN7* does not play an essential role in UV damage repair. One possibility is that Din7 participates in other DNA repair pathways that do not contribute directly to UV survival. Alternatively, it may play a role in UV damage repair but its loss does not affect survival rate due to redundancy of similar components.

The results shown in Figure 2A and B clearly demonstrate that *EXO1* expression is UV inducible. However, these data did not indicate if the damage inducibility of *EXO1* is specific for UV light, even though deletion of *EXO1* leads only to UV sensitivity and not sensitivity to γ-ray or MMS treatments. Because we found that the distribution of cells at various points in the cell cycle does not change dramatically following UV treatment (our unpublished observation), we also do not believe that inducibility of the *EXO1* gene is a result of cell cycle regulation.

Recently, functional analysis of the Exo1 enzyme has been carried out in several laboratories (13,17,25). Exonuclease-1 activity has been demonstrated to be highly inducible during meiosis in *S.pombe* and is involved in the late steps of homologous recombination, i.e. correction of mismatched base pairs resulting from hybrid DNA formation (13). It has also been strongly indicated by *in vitro* and *in vivo* experiments that *S.cerevisiae* Exo1 functions in mitotic recombination (17). *Sc*Exo1 also interacts with Msh2, a major recognition protein in the DNA mismatch repair pathway (32). Since Msh2 is also involved in recombination, it is not clear whether the Exo1–Msh2 complex identified is involved in MMR, recombination or both. However, it is reasonable to assume that the major function of *Sc*Exo1 nuclease is in MMR and recombination.

Our observation that *Sc*Exo1 functions in UV damage repair via a distinct pathway from NER raises an interesting question: is the observed effect of the *exo1* deletion mutation on UV resistance derived from the role of *EXO1* in recombination and/or MMR? In order to determine if there is a relationship between the UV resistance function of *EXO1* and its roles in recombination and MMR, an epistasis analysis with mutations that cause defects in recombination and MMR was employed. Our results indicate that while a recombination-defective *rad51* mutant and a MMR-deficient *msh2* mutant both display minor UV sensitivity, the *exo1 rad51* and *exo1 msh2* double mutants were both more UV sensitive than the corresponding single mutants. This suggests that the UV resistance mediated by *EXO1* may be separate from its roles in recombination and MMR. Interestingly, the *rad2 rad51* and *rad2 msh2* mutants were both more UV sensitive than the corresponding single mutants, indicating that the *RAD2*, *RAD51*, *MSH2* and *EXO1* genes all make distinct contributions to the UV resistance of *S.cerevisiae*. We speculate that this could represent the repair of distinct classes of DNA lesions or could represent distinct UV damage bypass mechanisms.

Table 3. UV survival of wild-type and mutant *S.cerevisiae* strains

UV dose	Strains (percent survival)[a]							
(J/m^2)	*wt*	*exo1*	*msh2*	*rad51*	*rad2*	*rad2exo1*	*rad2rad51*	*rad2msh2*
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	nd	nd	nd	nd	94.0	81.0	45.0	69.0
3	nd	nd	nd	nd	42.0	22.0	6.0	29.0
4	nd	nd	nd	nd	19.0	6.0	1.0	7.0
5	99.5	99.4	99.6	99.4	3.5	0.8	0.1	1.0

[a]Isogenic wild-type and mutant strains were grown to stationary phase and appropriate dilutions plated onto YPD agar prior to irradiaton and incubation at 30°C for 3–5 days in the dark. Percent survival was calculated as the number of cells surviving to form a colony after UV exposure divided by the number of irradiated cells (0 J/m^2) that could form colonies. nd, survival was not determined.

If the UV resistance function of Exo1 nuclease is not related to its roles in DNA recombination and MMR, what other possibilities should be considered? In the fission yeast *S.pombe*, an NER-independent UV damage repair pathway has been well defined. This pathway possesses a considerable capacity to remove CPDs and 6–4 PPs by a mechanism that is distinct from NER or base excision repair (BER) (8,9,34). The enzyme, named *S.pombe* DNA endonuclease (SPDE) or UV damage endonuclease (UVDE), has been isolated and characterized (11,35). Meanwhile, the *Sp*Rad2 nuclease has been proposed to be the second component of this pathway, acting as a 5′ flap endonuclease in removal of the adducted DNA fragment which was released by SPDE (36). The fact that similar synergistic effects are observed in both *S.cerevisiae rad2 exo1* and *S.pombe rad2 UVDE* double mutants (10,30) supports the notion that *S.cerevisiae* possesses a minor NER-independent pathway as well. However, it was indicated that photoproducts were not removed from DNA in *S.cerevisiae* NER mutants using antibodies directed against CPD and 6–4 PPs (37). Therefore, we must consider the possibility that residual UV resistance is the result of other mechanisms, such as error-prone repair, that permit DNA to bypass or shuffle damaged bases rather than excise them. Exo1 has been suggested to participate in RNA primer removal during lagging strand DNA synthesis in DNA replication (26; our unpublished data). It may play a role in the bypass of UV lesions by maintaining DNA replication in abnormal circumstances, such as after a high dosage of UV.

This work presents evidence that *Sc*Exo1 plays a role in UV resistance in addition to its roles in DNA recombination and mismatch repair (17,25). The information made available in this report will be useful in further functional analysis of the newly emerging family of nucleases in DNA replication, repair and recombination.

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