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ORIGINAL PAPER

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The *upr-1* gene encodes a catalytic subunit of the DNA polymerase ζ which is involved in damage-induced mutagenesis in *Neurospora crassa*

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Abstract The *upr-1* mutant was one of the first mutagen-sensitive mutants to be isolated in *Neurospora crassa*. However, the function of the *upr-1* gene has not yet been elucidated, although some genetic and biochemical data have been accumulated. In order to clone the *upr-1* gene, we performed a chromosome walk from the *mat* locus, the closest genetic marker to *upr-1* for which a molecular probe was available, towards the centromere, and a chromosomal contig of about 300–400 kb was constructed. Some of these clones complemented the temperature sensitivity of the *un-16* mutation, which is located between *mat* and *upr-1*. The *un-16* gene was sequenced, and localized in the MIPS *Neurospora crassa* genome database. We then searched the regions flanking *un-16* for homologs of known DNA repair genes, and found a gene homologous to the *REV3* gene of budding yeast. The phenotype of the *upr-1* mutant is similar to that of the yeast *rev3* mutant. An *ncrev3* mutant carrying mutations in the *N. crassa REV3* homolog was constructed using the RIP (repeat-induced point mutation) process. The spectrum of mutagen sensitivity of the *ncrev3* mutant was similar to that of the *upr-1* mutant. Complementation tests between the *upr-1* and *ncrev3* mutations indicated that the *upr-1* gene is in fact identical to the *ncrev3* gene. To clarify the role of the *upr-1* gene in DNA repair, the frequency of MMS and 4NQO-induced mutations was assayed using the *ad-8* reversion test. The *upr-1* mutant was about 10 times less sensitive to both chemicals than the wild type. The expression level of the *upr-1* gene is increased on exposure to UV irradiation in the *uvs-2* and *mus-8* mutants, which belong to postreplication repair group, as well as in the wild type. All these results suggest that the product of the *upr-1* gene functions in damage-induced mutagenesis and DNA translesion synthesis in *N. crassa*.

Keywords *Neurospora crassa* · *upr-1* · *REV3* · Mutagenesis · DNA repair

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Introduction

All organisms possess mechanisms for the recognition and repair of damaged DNA, which help to maintain the integrity of the genome. Mutants that are defective in these mechanisms show various phenotypes, such as high sensitivity to mutagens, abnormal frequencies of induced mutations, and unusual patterns of cell cycle progression. In the filamentous fungus *Neurospora crassa*, over 40 different mutagen-sensitive mutants have been isolated, and characterized both genetically and biochemically (Inoue 1999). Several epistasis groups of DNA repair genes have been identified, namely the *mus-38* epistasis group, involved in nucleotide excision repair (NER), the *mus-18* epistasis group, which specifies a second pathway for NER, the *uvs-2* epistasis group for postreplication repair (PRR), the *uvs-6* epistasis group for recombinational repair, and the *phr* group which mediates photoreactivation repair. Functions have not yet been assigned to two other epistasis groups, represented by the *uvs-3* and *upr-1* genes, respectively.

The *upr-1* epistasis group contains the genes *upr-1* and *mus-26*. Both mutants are sensitive to ultraviolet light (UV) and 4-nitroquinoline-1-oxide (4NQO), but less sensitive to γ-rays, methylmethane sulfonate (MMS) and mitomycin C (MMC). They are also partially defective in photoreactivation and less mutable upon exposure to UV irradiation (Tuveson and Mangan 1970; Inoue and Ishii 1985; Ishii and Inoue 1989).

In order to analyze the *upr-1* gene at the molecular level, we attempted to isolate the gene by complementation with a *N. crassa* genomic library, but this endeavor ended in failure. As a alternative approach to

cloning of the gene, we began a chromosomal walk from a cloned gene located near the *upr-1* gene. We localized the *un-16* gene, which maps close to the *upr-1* gene, in the walk, and sequenced it. Then, we searched for DNA repair genes in the neighborhood of the *un-16* gene using the *N. crassa* genomic sequence database, and found a homolog of the budding yeast *REV3* gene. The phenotype of the *upr-1* mutant is similar to that of the yeast *rev3* mutant and the *Aspergillus nidulans uvsI* mutant (a *rev3* homolog; Haynes and Kunz 1981; Chae and Kafer 1993; Han et al. 1998).

In this paper we constructed and characterized a strain that was mutant for the *N. crassa REV3* homolog, and demonstrated that the gene is identical to *upr-1*. We also present evidence to show that the *upr-1* gene product functions in damage-induced mutagenesis.

Materials and methods

Strains and plasmids

The *N. crassa* strains used in this study are listed in Table 1. The *Escherichia coli* strains DH-1 and XL-1 Blue were used for amplification of plasmids. pBluescript SK^+ (Stratagene) was used for general DNA manipulation. pCSN44 (Staben et al. 1989), carrying the *E. coli* hygromycin B resistance gene driven by the *A. nidulans trpC* promoter, was used as a vector for transformation of *N. crassa* cells. For induction of the RIP phenomenon (Selker et al. 1987), the plasmid pR3-S was constructed by inserting a 1.6-kb *Sac*I DNA fragment of the pMOcosX cosmid clone X3:C7 into pCSN44. The *Sac*I fragment encodes conserved DNA polymerase motifs described by Wong et al. (1988). The plasmid pNCREV3/*Eco*RV was constructed by inserting the 11-kb *Eco*RV fragment of X3:C7 into the *Eco*RV site of pCSN44, and used for transformation of the *upr-1* mutant.

General genetic manipulations in *N. crassa*

General genetic manipulations, including crosses and complementation testing of *N. crassa*, were carried out according to the methods of Davis and de Serres (1970). Transformation of *N. crassa* cells was performed as described by Vollmer and Yanofsky (1986) and Tomita et al. (1993), except that the lysing enzyme from *Trichoderma harzianum* (Sigma) was used (at a concentration of 2 mg/ml in 1 M sorbitol) to produce spheroplasts. The *ncrev3* mutant was generated by RIPping, as described previously (Watanabe et al. 1997; Shimura et al. 1999).

DNA and RNA manipulations

Standard molecular techniques were carried out according to Sambrook et al. (1989). DNA sequencing was carried out using the ALFexpress sequencer (Amersham Pharmacia Biotech). *N. crassa* RNA was isolated from germinating conidia (final concentration 1×10^7/ml), which had been cultured for 6 h at room temperature with shaking. Total RNAs were extracted by using RNAwiz (Ambion). The *Sac*I DNA fragment from pR3-S was labeled with ^{32}P using the Multiprime DNA labeling system (Amersham Pharmacia Biotech) and used as a DNA probe for Northern hybridization analysis.

Chromosome walking

Chromosome walking was carried out using two wild-type *N. crassa* genomic libraries, constructed in the cosmid vector pMOcosX (Orbach 1994) and in pSV50 (Vollmer and Yanofsky 1986). These libraries were obtained from the Fungal Genetics Stock Center (FGSC), Department of Microbiology, University of Kansas Medical School, Kansas City, Kan. Chromosome walking was performed in one direction by using end fragments as probes for each step. Selected cosmid were physically mapped by RFLP analysis (Metzenberg et al. 1985).

Mutagen sensitivity and photoreactivation

N. crassa conidia were irradiated with UV, or treated with 4NQO or MMS, and the survival rate was determined as described previously (Inoue and Ishii 1984). The assay for photoreactivation was carried out as described by Inoue and Ishii (1985).

Frequency of mutagen-induced reversion at the *ad-8* locus

The indicated amounts of chemical mutagen were added directly to a suspension of *ad-8* conidia (2×10^7 conidia/ml) and the suspension was incubated at 30°C for 2 h with gentle shaking. The conidia were then collected and washed twice by centrifugation.

To measure the surviving fraction, a portion of the conidial suspension was diluted to 10^4 conidia/ml, and 0.1-ml samples of the

Table 1 Strains of *Neurospora crassa* used in this study

Strain type	Strain number	Genotype	Source/reference
Wild type	C1-T10-37A	*A*	Laboratory stock[a]
	C1-T10-28a	*a*	Laboratory stock[a]
	74-OR31-16A	*A al-2 pan-2 cot-1*	De Serres et al. (1980)
	C3-T28-13A	*A al-2 pan-2 cot-1 ad-8*	Kimura et al. (1986)
upr-1	74-OR256-2A	*A al-2 pan-2 cot-1 upr-1*	De Serres et al. (1980)
	C3-T46-1A	*A al-2 pan-2 cot-1 ad-8 upr-1*	Ishii and Inoue (1989)
ncrev3	9-1-6	*A upr-1^{RIP}*	This study[b]
	9-1-6-5	*A nic-3 upr-1^{RIP}*	This study[c]
nic-3	FGSC 4081	*A nic-3*	FGSC
	FGSC 4082	*a nic-3*	FGSC
uvs-2	74-OR244-3A	*A al-2 pan-2 cot-1 uvs-2*	De Serres et al. (1980)
mus-8	FGSC 5144	*A mus-8*	FGSC
un-16	FGSC 4306	*A un-16*	FGSC

[a]Derivative of 74-OR31-16A (wild type) × FGSC 988a (wild type). FGSC, Fungal Genetic Stock Center
[b]The *upr-1^{RIP}* mutation was generated by the RIP process
[c]Derivative of FGSC 4082 (*nic-3 a*) × 9-1-6 (*upr-1^{RIP} A*)

diluted suspension were mixed with medium supplemented with adenine sulfate (100 μg/ml) and panthotenate (10 μg/ml) and plated on petri dishes. Each plate was incubated at 30°C for 2 days and the numbers of colonies were counted.

To determine the number of *ad-8* revertants, 1-ml aliquots of the conidial suspension was mixed with medium supplemented with adenine sulfate (0.1 μg/ml) and panthotenate (10 μg/ml) and plated on petri dishes. The number of revertants was counted after incubation for 3 days. Reversion frequency is expressed as the number of revertants/10^8 survivors.

Results

Chromosome walking

A genetic map of the region around the *upr-1* gene is shown in Fig. 1. The *upr-1* gene maps on the left arm of linkage group I, near the *mat* locus. In order to isolate the *upr-1* gene, we started a chromosome walking from cosmid 6:10A, which contains the *mat* gene (Glass et al. 1988). In the first screen, four cosmid clones were found to hybridize to cosmid 6:10A. One of these, 17:2B, had already been mapped by RFLP to the centromere-proximal side of the *mat* locus (Perkins et al. 2001). After several walking steps, a total of 53 independent cosmid clones were identified (Table 2), and a contig consisting of 16 clones was constructed, which covers a genomic region of about 300–400 kb.

Three cosmids, G11:E9, X3:C7 and X8:A3, complemented the temperature sensitive phenotype associated with the *un-16* mutation, which maps between *mat* and *upr-1* (Perkins et al. 2001). Sequence analysis of the smallest DNA fragment which complemented the *un-16* mutation revealed that the *un-16* gene encodes the homolog of *Podospora anserina* ribosomal protein S9 gene (data not shown). We then searched for homologs of DNA repair genes in the region around the *un-16* gene, using the MIPS *Neurospora crassa* genomic database.

Characterization of the *N. crassa REV3* homolog

Contig B2G21 in the MIPS database includes the *un-16* gene. Analysis of this contig sequence showed that a homolog of the budding yeast *REV3* gene, which encodes a catalytic subunit of DNA polymerase ζ (polζ), is present in the same contig. The *REV3* homolog was temporarily designated as *ncrev3*. The protein encoded by the *ncrev3* gene is predicted to contain 1926 amino acids, while the yeast protein contains 1504 amino acids (Morrison et al. 1989). The result of a comparison, using Clustal-W 1.8 algorithm, of the NCREV3 protein with the yeast Rev3 protein is schematically represented in Fig. 2. The degree of sequence conservation of the NCREV3 protein relative to its yeast counterpart within the N-terminal region was considerable (29% identity, 48% similarity). Extensive homology (44% identity and 62% similarity) was found in the region of the C-terminus, which contains the six conserved DNA polymerase motifs defined by Wong et al. 1988). Small region adjacent to each of the six DNA polymerase motifs in the C-terminal domain was also highly conserved (33% identity, 45% similarity).

Table 2 Chromosome walking from the *mat* locus

Cosmid clones used as probes	Identified cosmid clones
6:10A[a]	6:3C, 13:12E, 17:2B, 21:4E
17:2B	G11:E9, X25:F2
G11:E9	G5:C1
G5:C1	X3:C7, X8:A3, X9:B11, X17:G13, X22:F2
X9:B11	G3:C8, G4:D11, G12:C6, G21:G2, G24:H3, G10:C4, X15:H11, X20:E5
G4:D11	G13:H12, X13:F9, X14:A10, X19:C2, X21:G10
X21:G10	G6:D8
G6:D8	X10:C3, X15:B10
X15:B10	G2:C4, G12:C2, X3:G4, X5:C5, X6:E12, X7:G4, X7:H4
X3:G4	G18:F7, X18:D2, X18:C11, X25:D3
X18:D2	X14:B2, X21:E4
X14:B2	X17:B10
X17:B10	X14:D9
X14:D9	G2:D8, G3:A3, G17:F11, G21:F4, G21:F6, G25:D2, X16:B6
X16:B6	X11:C4, X13:D9, X15:G5

[a]Cosmid 6:10A contains the *mat* gene (Glass et al. 1988). Chromosome walking was carried out using the pMOcosX library; the initiating clone was isolated from a genomic library constructed in pSV50. The cosmid clones used as the probes to extend the walk are *underlined*

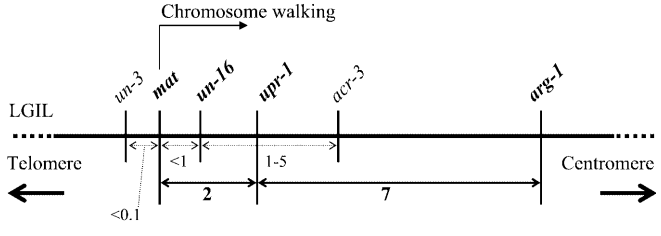

Fig. 1 Partial genetic map of linkage group I left (LGIL) showing the *upr-1* gene and nearby genes (Perkins et al. 2001). The *upr-1* locus is located between the *mat* (2 map units away) and *arg-1* (7 m.u. away) loci. Chromosome walking was started from the cosmid clone 6:10A (isolated from a pSV50 genomic library) containing the *mat* gene and extended towards the centromere

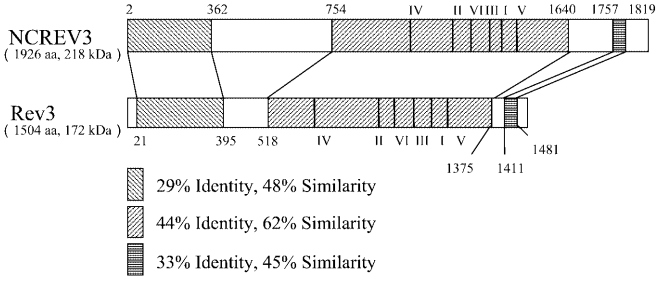

Fig. 2 Schematic comparison between the putative *N. crassa* protein NCREV3 and *S. cerevisiae* Rev3. The *Roman numerals* refer to motifs conserved in DNA polymerases (Wong et al. 1998)

Complementation tests of the *upr-1* and *ncrev3* mutants

The *ncrev3* gene lies near the *un-16* gene, which itself is known to be close to the *upr-1* gene. Furthermore, the phenotype of the *upr-1* mutant (Ishii and Inoue 1989) is similar to that of the yeast *rev3* and *A. nidulans uvsI* mutants; the *upr-1*, *rev3* and *uvsI* mutants are all sensitive to UV and/or 4NQO, and less sensitive to MMS (Haynes and Kunz 1981; Chae and Kafer 1993; Han et al. 1998). These mutants also exhibit low UV-induced mutability (Lawrence and Christensen 1976; Chae and Kafer 1997). These similarities raised the possibility that the *upr-1* and *ncrev3* genes might be identical.

In order to test this, we generated an *ncrev3* mutant by RIPping (Selker et al. 1987). The mutagen sensitivity of the *ncrev3* mutant was assessed by a spot test (Fig. 3). The spectrum of mutagen sensitivity of the *ncrev3* mutant was similar to that of the *upr-1* mutant. Dose-response curves constructed following exposure of the *upr-1* and *ncrev3* mutants to UV showed a characteristic pattern with a shoulder around 100 J/m^2 (Fig. 4). When both strains were transformed with the cosmid X3:C7 containing the *ncrev3* gene, they became as resistant to UV as the wild type (Fig. 3). Another transformation experiment, using pNCREV3/*Eco*RV, which contains an 11-kb *Eco*RV fragment derived from X3:C7 (Fig. 5), gave the same results as with X3:C7 (data not shown). To further confirm these results, we constructed a forced heterokaryon strain that contained two types of nuclei, i.e. one carrying the *upr-1* mutation and another carrying the *ncrev3* mutation. The resulting heterokaryon strain was sensitive to UV, indicating that the two mutations could not complement each other (Fig. 6).

It is known that the *upr-1* mutant shows a partial photoreactivation defect (PPD) (Tuveson and Mangan 1970). Figure 7 shows that the level of photoreactivation achieved after UV irradiation was much lower in the *ncrev3* mutant than in the wild type. In the wild type (Fig. 7A), the viability of UV-irradiated conidia showed a rapid recovery within the first 10 min of photoreactivation, and then the incremental rate of recovery gradually fell, and finally saturated after 30 min. Although the *ncrev3* mutant (Fig. 7B) showed some recovery of the cells by photoreactivation, the incremental rate was very low and saturated earlier than in the wild type at a low level of viability.

Taken together, these results indicate that the *N. crassa REV3* homolog (*ncrev3*) is identical to the *upr-1* gene. The Genbank Accession No. for the *upr-1* gene is AB077040.

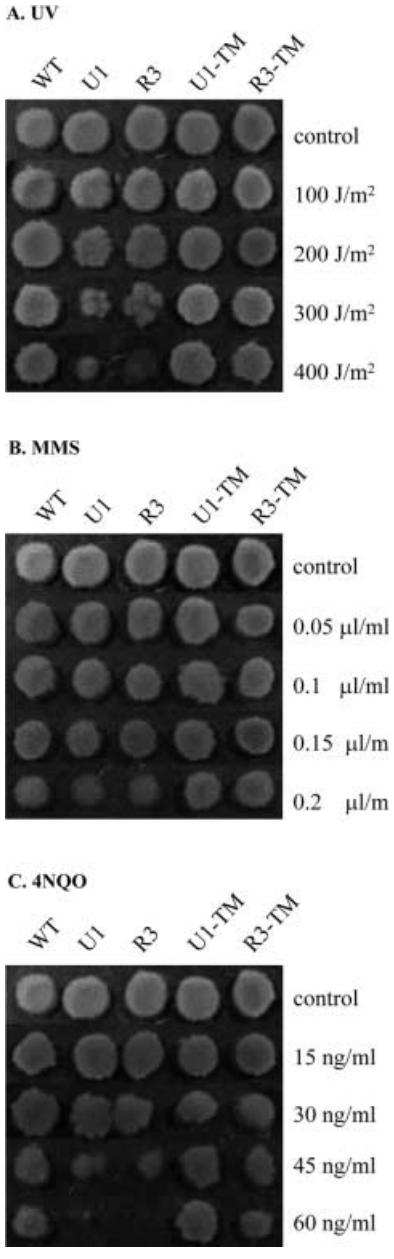

Fig. 3A–C Sensitivity of the wild type, *upr-1* and *ncrev3* mutants and their transformants to UV (**A**), MMS (**B**), and 4NQO (**C**). The wild-type strain (C1-T10-37A; WT), and the *upr-1* (74-OR256-2A; U1) and *ncrev3* (9-1-6; R3) mutants, a *upr-1* transformant (U1-TM) and a *ncrev3* transformant (R3-TM) were cultured in the presence of the dose or concentration of the mutagen indicated to the *right* of each panel

Characterization of the *upr-1* gene product as an error-prone DNA polymerase

The *upr-1* mutant is 3–4 times less mutable than the wild type upon exposure to UV irradiation (Ishii and Inoue 1989). In the experiment reported here, reversion frequencies induced by two chemicals, MMS and 4NQO, were measured. As shown in Fig. 8, the *upr-1* mutant was about 10 times less mutable to MMS and 4NQO than the wild type, in good agreement with the effect observed in the case of UV irradiation.

Expression of *upr-1* after UV irradiation

Conidia of the wild-type, *uvs-2* and *mus-8* strains were cultured for 6 h at room temperature. Then they were irradiated with UV light (100 J/m^2) and further cultured

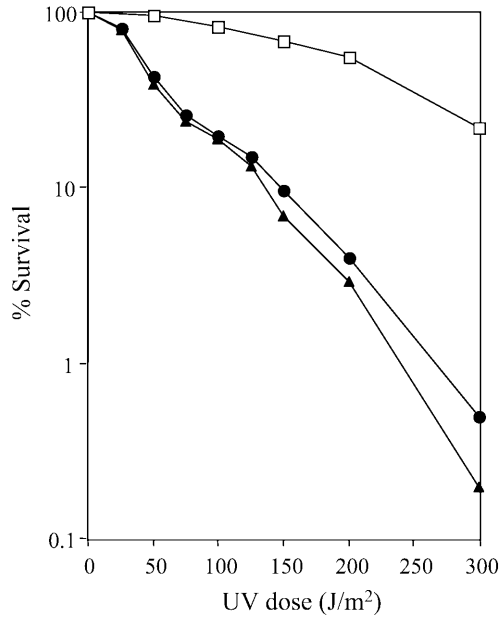

Fig. 4 Dose-response curves of the wild type, *upr-1* and *ncrev3* mutants on exposure to UV irradiation. Each point represents the mean of at least three independent experiments. *Open squares*, wild type (C1-T10-37A); *filled circles*, *upr-1* (74-OR256-2A); *filled triangles*, *ncrev3* (9-1-6)

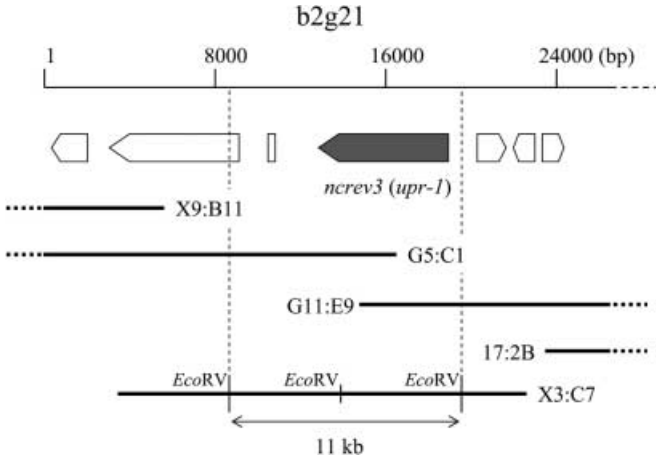

Fig. 5 Relationship between the contig B2G21 from the MIPS *N. crassa* database and some of the cosmids isolated in our chromosome walking. The *arrowed boxes* indicate hypothetical genes. The plasmid pNCREV3/*Eco*RV, used for complementation testing, was constructed by inserting the indicated 11-kb *Eco*RV fragment of cosmid X3:C7 into pCSN44

for the various times. Total RNA was isolated from the germinated conidia and used for Northern analysis with the *upr-1* gene as a probe. As shown in Fig. 9, the *upr-1* transcript was not detected in the unirradiated condition (Fig. 9), but it appeared by 15 min after UV irradiation. The transcript level attained its maximum 30 min after UV irradiation and then decreased gradually in the wild type. There was no significant difference in the induction pattern between the wild type and the *uvs-2* or *mus-8* mutant, although high-level expression lasted for over

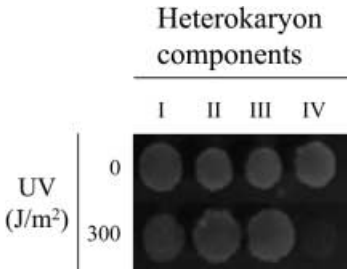

Fig. 6 UV sensitivity of four different forced heterokaryons. The forced heterokaryons have the following genotypes: *pan-2 A* and *nic-3 A* (Het I); *pan-2 upr-1 A* and *nic-3 A* (Het II); *pan-2 A* and *nic-3 ncrev3 A* (Het III); *pan-2 upr-1 A* and *nic-3 ncrev3 A* (Het IV) and were exposed to the indicated dose of UV

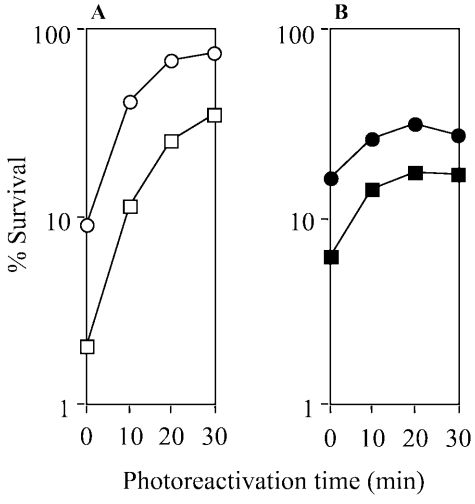

Fig. 7A, B Effects of photoreactivation in the wild type and *ncrev3* mutant. **A** Conidial suspensions of the wild-type strain (74-OR31-16A) were UV-irradiated with doses of 350 J/m^2 (*open circles*) and 500 J/m^2 (*open squares*) and immediately placed under visible light for the indicated time to induce photoreactivation. **B** Conidial suspensions of the *ncrev3* (9-1-6) mutant were UV-irradiated with doses of 100 J/m^2 (*filled circles*) and 175 J/m^2 (*filled squares*) and photoreacted. Each point represents the mean of at least three independent experiments

60 min after UV irradiation in the *uvs-2* mutant and expression was generally lower in the *mus-8* mutant.

Discussion

The *uvs-2* and *mus-8* genes of *N. crassa* are homologs of the *S. cerevisiae* genes *RAD18* and *RAD6*, respectively (Tomita et al. 1993; Soshi et al. 1996). In earlier studies, *upr-1* was placed in the *uvs-2* epistasis group for the following reasons. (1) The *upr-1* mutant was normal in homozygous crosses (Tuveson 1972), and there was no difference between the wild type and the *upr-1* mutant with respect to the spontaneous mutation frequency (de Serres et al. 1980). (2) The *upr-1* mutant showed increased UV- and γ-ray-induced mutation frequencies in the *ad-3* forward mutation test (de Serres 1980;

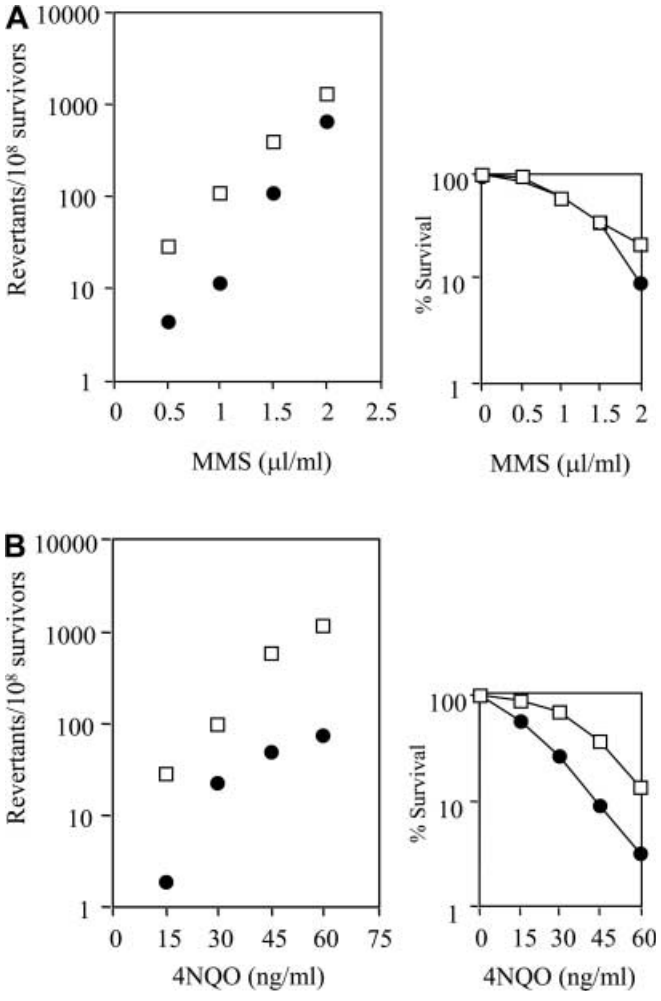

Fig. 8A, B Frequency of mutagen-induced reversion at the *ad-8* locus. Cells were treated with MMS (**A**) or 4NQO (**B**) to induce reversion. The panels on the *left* show the frequency of adenine auxotrophs per 10^8 survivors. In the panels on the *right*, the survival rate is plotted against the mutagen concentration. *Open squares*, wild type (C3-T28-13A); *filled circles*, *upr-1* (C3-T46-–1A)

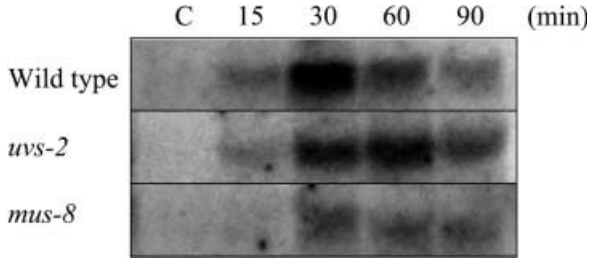

Fig. 9 Northern analysis of the *upr-1* transcript in the wild type, and *uvs-2* and *mus-8* mutants. Germinating conidia were irradiated with UV at a dose of 100 J/m^2. At indicated time after irradiation, samples were collected and total RNA was isolated. Aliquots (30 μg) of total RNA were fractionated by electrophoresis, and Northern hybridization analysis was carried out using the *upr-1* gene as a probe

Schupbach and de Serres 1981). (3) The *upr-1* mutant could not excise pyrimidine dimers (Worthy and Epler 1973). These phenotypes were similar to those of the *uvs-2* mutant. However, later data do not agree with this assignment. (1) Both *upr-1* and *uvs-2* mutants can excise pyrimidine dimers (Baker et al. 1990, 1991). (2) Double mutants carrying the *upr-1* and *uvs-2* mutations showed slightly higher UV sensitivity than either of the single mutants (Ishii and Inoue 1989). (3) In the *ad-8* reversion test, the *upr-1* mutant showed a reduced mutation frequency, whereas the *uvs-2* mutant showed an increased mutation frequency (Ishii and Inoue 1989). Thus, it appears that *upr-1* belongs to a separate group from the *uvs-2* epistasis group, or alternatively, the *upr-1* epistasis group may possibly represent a subgroup of the *uvs-2* epistasis group (Schroeder et al. 1998).

In this study, our results indicate that the *upr-1* gene is a homolog of the *S. cerevisiae REV3* gene, which encodes a catalytic subunit of polζ (Morrison et al. 1989). Polζ is known as an error-prone DNA polymerase which functions in DNA translesion synthesis (TLS; Nelson et al. 1996). The yeast *rev3* mutant shows decreased mutability (Kunz et al. 2000). In the experiment using the *N. crassa ad-8* reversion test, the *upr-1* mutant showed low induced mutability not only following UV irradiation but also in response to MMS and 4NQO treatments. These results indicate that the *upr-1* gene product functions in damage-induced mutagenesis as an error-prone DNA polymerase.

It has been suggested that the *RAD6* DNA repair pathway in yeast actually consists of two parallel subpathways, i.e. error-free and error-prone pathways (Xiao et al. 1999). The *REV3* gene is known to function in the *RAD6* error-prone pathway (Friedberg 1991). In *N. crassa*, the *uvs-2* group is the counterpart of the yeast *RAD6* group. To examine whether the expression of the *upr-1* gene is regulated by the *uvs-2* and/or *mus-8* gene products, we measured the amounts of UV-induced *upr-1* transcript present in the wild type and *uvs-2* and *mus-8* mutants. Our results showed that expression of *upr-1* is inducible by UV, and the *upr-1* transcript appeared within 15 min after UV irradiation in the wild type. This response is faster than that observed with other *N. crassa* DNA repair genes, i.e. *mus-23*, *mus-25* and *mus-11* (Watanabe et al. 1997; Handa et al. 2000; Sakuraba et al. 2000), suggesting that the *upr-1* gene product functions at an early stage of the response to UV irradiation. In the *uvs-2* and *mus-8* mutants, the pattern of *upr-1* expression was similar to that in the wild type, though expression in the *mus-8* mutant was somewhat low. Therefore, *upr-1* expression is not severely affected by the lack of either the *uvs-2* or the *mus-8* gene. The *uvs-2* mutant shows an increased *ad-8* reversion frequency (Ishii and Inoue 1989). The *upr-1* gene product might be responsible for the high mutability observed in the *uvs-2* mutant.

The *upr-1* and *mus-26* mutants both show a partial photoreactivation defect (PPD) after UV irradiation (Tuveson and Mangan 1970, Ishii and Inoue 1989). Why the *upr-1* and *mus-26* mutants show the PPD phenotype, although both have normal cyclobutane pyrimidine dimer (CPD) photolyase activity (our unpublished data), has remained unclear for a long time. The results

presented in this study indicate that the PPD phenotype is due to the defect in the *upr-1* gene product, polζ. In UV-irradiated DNA, (6-4) photoproducts are formed much less frequently than cyclobutane pyrimidine dimers (CPDs) (Friedberg et al. 1995). CPDs are repaired efficiently by CPD photolyase during photoreactivation. Some organisms, such as *Drosophila meranogaster*, have a (6-4) photolyase which splits (6-4) photoproducts (Todo et al. 1993). *N. crassa* has no (6-4) photolyase activity (Shimura et al. 1999), indicating that (6-4) photoproducts are removed by the dark repair systems in *N. crassa*. When the replication machinery encounters (6-4) photoproducts during replication, polζ and other TLS polymerase(s) might bypass these lesions efficiently in the wild type, but not in the *upr-1* mutant. This suggests that the PPD phenotype of the *upr-1* mutant is caused by the lack of efficient bypass of (6-4) photoproducts by the TLS function. This model can also be applied to the PPD phenotype conferred by mutations in the *mus-38* gene, which is a homolog of the *S. cerevisiae RAD1* gene. The *mus-38* mutant shows the PPD phenotype in addition to defects in NER (Hatakeyama et al. 1998; Ishii et al. 1998). So the PPD phenotype might be observed when either removal (*mus-38*) or bypass (*upr-1*) of (6-4) photoproducts is defective. Recent reports have proposed that the combined activity of eukaryotic polι and polζ coordinates the bypass of (6-4) photoproducts (Johnson et al. 2000; Tissier et al. 2000). Others have suggested that the sequential activity of yeast polη and polζ could coordinate the bypass of (6-4) photoproducts (Johnson et al. 2001; Yu et al. 2001). These suggestions are not in conflict with our model. To confirm our model, we are currently characterizing mutants defective in *N. crassa* genes homologous to yeast *REV1* and *REV7*.

References

Baker TI, Cords CE, Howard CA, Radloff RJ (1990) The nucleotide excision repair epistasis group in *Neurospora crassa*. Curr Genet 18:207–209

Baker TI, Radloff RJ, Cords CE, Engel SR, Mitchell DL (1991) The induction and repair of (6-4) photoproducts in *Neurospora crassa*. Mutat Res 255:211–218

Chae SK, Kafer E (1993) *uvsI* mutants defective in UV mutagenesis define a fourth epistatic group of *uvs* genes in *Aspergillus*. Curr Genet 24:67–74

Chae SK, Kafer E (1997) Two *uvs* genes of *Aspergillus nidulans* with different functions in error-prone repair: *uvsI*, active in mutation-specific reversion, and *uvsC*, a *recA* homolog, required for all UV mutagenesis. Mol Gen Genet 254:643–653

Davis RH, de Serres FJ (1970) Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol 17:79–143

De Serres FJ (1980) Mutagenesis at the *ad-3A* and *ad-3B* loci in haploid UV-sensitive strains of *Neurospora crassa*. II. Comparison of dose-response curves for inactivation and mutation induced by UV. Mutat Res 71:181–191

De Serres FJ, Inoue H, Schupbach ME (1980) Mutagenesis at the *ad-3A* and *ad-3B* loci in haploid UV-sensitive strains of *Neurospora crassa*. I. Development of isogenic strains and spontaneous mutability. Mutat Res 71:53–65

Friedberg EC (1991) Yeast genes involved in DNA-repair processes: new looks on old faces. Mol Microbiol 5:2303–2310

Friedberg EC, Walker GC, Siede W (1995) DNA repair and mutagenesis. ASM Press, Washington, D.C.

Glass NL, Vollmer SJ, Staben C, Grotelueschen J, Metzenberg RL, Yanofsky C (1988) DNAs of the two mating-type alleles of *Neurospora crassa* are highly dissimilar. Science 241:570–573

Han KY, Chae SK, Han DM (1998) The *uvsI* gene of *Aspergillus nidulans* required for UV-mutagenesis encodes a homolog to *REV3*, a subunit of the DNA polymerase ζ of yeast involved in translesion DNA synthesis. FEMS Microbiol Lett 164:13–19

Handa N, Noguchi Y, Sakuraba Y, Ballario P, Macino G, Fujimoto N, Ishii C, Inoue H (2000) Characterization of the *Neurospora crassa mus-25* mutant: the gene encodes a protein which is homologous to the *Saccharomyces cerevisiae* Rad54 protein. Mol Gen Genet 264:154–163

Hatakeyama S, Ito Y, Shimane A, Ishii C, Inoue H (1998) Cloning and characterization of the yeast *RAD1* homolog gene (*mus-38*) from *Neurospora crassa*: evidence for involvement in nucleotide excision repair. Curr Genet 33:276–283

Haynes RH, Kunz BA (1981) DNA repair and mutagenesis in yeast. In: Strathern JN, Jones EW, Broach JR (eds) The molecular biology of the yeast *Saccharomyces*. Life cycle and inheritance. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, pp 371–414

Inoue H (1999) DNA repair and specific-locus mutagenesis in *Neurospora crassa*. Mutat Res 437:121–133

Inoue H, Ishii C (1984) Isolation and characterization of MMS-sensitive mutants of *Neurospora crassa*. Mutat Res 125:185–194

Inoue H, Ishii C (1985) A new ultraviolet-sensitive mutant of *Neurospora crassa* with unusual photoreactivation property. Mutat Res 152:161–168

Ishii C, Inoue H (1989) Epistasis, photoreactivation and mutagen sensitivity of DNA repair mutants *upr-1* and *mus-26* in *Neurospora crassa*. Mutat Res 218:95–103

Ishii C, Nakamura K, Inoue H (1998) A new UV-sensitive mutant that suggests a second excision repair pathway in *Neurospora crassa*. Mutat Res 408:171–182

Johnson RE, Washington MT, Haracska L, Prakash S, Prakash L (2000) Eukaryotic polymerase ι and ζ act sequentially to bypass DNA lesions. Nature 406:1015–1019

Johnson RE, Haracska L, Prakash S, Prakash L (2001) Role of DNA polymerase η in the bypass of a (6-4) TT photoproduct. Mol Cell Biol 21:3558–3563

Kimura M, Osawa N, Ishii C, Inoue H (1986) Construction of testers for reversion assay. Neurospora Newslett 33:28–29

Kunz BA, Straffon AFL, Vonarx EJ (2000) DNA damage-induced mutation: tolerance via translesion synthesis. Mutat Res 451:169–185

Lawrence CW, Christensen RB (1976) UV mutagenesis in radiation-sensitive strains of yeast. Genetics 82:207–232

Metzenberg RL, Stevens JN, Selker EU, Morzycka-Wroblewska E (1985) Identification and chromosomal distribution of 5S rRNA genes in *Neurospora crassa*. Proc Natl Acad Sci USA 82:2067–2071

Morrison A, Christensen RB, Alley J, Beck AK, Bernstine EG, Lemontt JF, Lawrence CW (1989) *REV3*, a *Saccharomyces cerevisiae* gene whose function is required for induced mutagenesis, is predicted to encode a nonessential DNA polymerase. J Bacteriol 171:5659–5667

Nelson JR, Lawrence CW, Hinkle DC (1996) Thymine-thymine dimer bypass by yeast DNA polymerase ζ. Science 272:1646–1649

Orbach MJ (1994) A cosmid with a Hyg^R marker for fungal library construction and screening. Gene 150:159–162

Perkins DD, Radford A, Sachs MS (2001) The *Neurospora* compendium. Academic Press, San Diego

Sakuraba Y, Schroeder AL, Ishii C, Inoue H (2000) A *Neurospora* double-strand-break repair gene, *mus-11*, encodes a *RAD52* homologue and is inducible by mutagens. Mol Gen Genet 264:392–401

Sambrook J, Fritsch EF, Maniatis TM (1989) Molecular cloning: a laboratory manual (2nd edn). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Schroeder AL, Inoue H, Sachs MS (1998) DNA repair in *Neurospora*. In: Nickoloff JA, Hoekstra MF (eds) DNA repair in prokaryotes and lower eukaryotes (DNA damage and repair, vol 1). Humana Press, Totowa, N.J., pp 503–538

Schupbach ME, de Serres FJ (1981) Mutagenesis at the *ad-3A* and *ad-3B* loci in haploid UV-sensitive strains of *Neurospora crassa*. III. Comparison of dose-response curves for inactivation and mutation induced by γ-rays . Mutat Res 81:49–58

Selker EU, Cambareri EB, Jensen BC, Haack KR (1987) Rearrangement of duplicated DNA in specialized cells of *Neurospora*. Cell 51:741–752

Shimura M, Ito Y, Ishii C, Yajima H, Linden H, Harashima T, Yasui A, Inoue H (1999) Characterization of a *Neurospora crassa* photolyase-deficient mutant generated by repeat induced point mutation of the *phr* gene. Fungal Genet Biol 28:12–20

Soshi T, Sakuraba Y, Kafer E, Inoue H (1996) The *mus-8* gene of *Neurospora crassa* encodes a structural and functional homolog of the Rad6 protein of *Saccharomyces cerevisiae*. Curr Genet 30:224–231

Staben C, Jensen B, Singer M, Pollock J, Schechtman M, Kinsey J, Selker EU (1989) Use of a bacterial hygromycin B resistance gene as a dominant selectable marker in *Neurospora crassa* transformation. Fungal Genet Newslett 36:79–81

Todo T, Takemori H, Ryo H, Ihara M, Matsunaga T, Nikaido O, Sato K, Nomura T (1993) A new photoreactivating enzyme that specifically repairs ultraviolet light-induced (6-4) photoproducts. Nature 361:371–374

Tissier A, Frank EG, McDonald JP, Iwai S, Hanaoka F, Woodgate R (2000) Misinsertion and bypass of thymine-thymine dimers by human DNA polymerase ι. EMBO J 19:5259–5266

Tomita H, Soshi T, Inoue H (1993) The *Neurospora uvs-2* gene encodes a protein which has homology to yeast Rad18, with unique zinc finger motifs. Mol Gen Genet 238:225–233

Tuveson RW (1972) Genetic and enzymatic analysis of a gene controlling UV sensitivity in *Neurospora crassa*. Mutat Res 15:411–424

Tuveson RW, Mangan J (1970) A UV-sensitive mutant of *Neurospora* defective for photoreactivation. Mutat Res 9:455–466

Vollmer SJ, Yanofsky C (1986) Efficient cloning of genes of *Neurospora crassa*. Proc Natl Acad Sci USA 83:4869–4873

Watanabe K, Sakuraba Y, Inoue H (1997) Genetic and molecular characterization of *Neurospora crassa mus-23*: a gene involved in recombinational repair. Mol Gen Genet 256:436–445

Wong SW, Wahl AF, Yuan PM, Arai N, Pearson BE, Arai K, Korn D, Hunkapiller MW, Wang TS (1988) Human DNA polymerase α gene expression is cell proliferation dependent and its primary structure is similar to both prokaryotic and eukaryotic replicative DNA polymerases. EMBO J 7:37–47

Worthy TE, Epler JL (1973) Biochemical basis of radiation-sensitivity in mutants of *Neurospora crassa*. Mutat Res 19:167–173

Xiao W, Chow BL, Fontanie T, Ma L, Bacchetti S, Hryciw T, Broomfield S (1999) Genetic interactions between error-prone and error-free posteoreplication repair pathways in *Saccharomyces cerevisiae*. Mutat Res 435:1–11

Yu SL, Johnson RE, Prakash S, Prakash L (2001) Requirement of DNA polymerase η for error-free bypass of UV-induced CC and TC photoproducts. Mol Cell Biol 21:185–188