Repair of Damaged and Mismatched DNA by the XPC Homologues Rhp41 and Rhp42 of Fission Yeast

Thomas M. Marti,* Christophe Kunz† and Oliver Fleck*,[1]

**Institute of Cell Biology, University of Bern, CH-3012 Bern, Switzerland and* †*Institute of Medical Radiobiology, University of Zürich, CH-8008 Zürich, Switzerland*

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ABSTRACT

Rhp41 and Rhp42 of *Schizosaccharomyces pombe* are homologues of human XPC, which is involved in nucleotide excision repair (NER) of damaged DNA. Inactivation of *rhp41* caused moderate sensitivity to ultraviolet (UV) radiation. In addition, an increase of mitotic mutation rates was observed in the *rhp41* mutant, which was dependent on active translesion polymerase Z. UV sensitivity and mutation rates were not different between *rhp42* and wild type, but compared to *rhp41* were further increased in *rhp41 rhp42* cells. Transcription of the *fbp1* gene (induced in vegetative cells) and of the SPBC1289.14 gene (induced during meiosis) was strongly blocked by UV-induced damages in the *rhp41* mutant, but not, or only slightly, reduced in *rhp42* background. NER-dependent short-patch repair of mismatches formed during meiosis was slightly affected in *rhp41*, moderately affected in *rhp42*, and absent in *rhp41 rhp42*. Epistasis analysis with *rhp7* and *rhp26* indicates that Rhp41 and Rhp42 are both involved in the global genome and transcription-coupled repair subpathways of NER. Rhp41 plays a major role in damage repair and Rhp42 in mismatch repair.

NUCLEOTIDE excision repair (NER) is directed to a wide variety of DNA damages, including photoproducts induced by ultraviolet (UV) radiation (Petit and Sancar 1999). NER consists of two subpathways, namely transcription-coupled repair (TCR), which selectively removes damages from the transcribed strand of active genes, and global genome repair (GGR), which eliminates damages in nontranscribed DNA (Friedberg 2001; Hoeijmakers 2001).

The NER reaction occurs by damage recognition, dual strand incision, DNA synthesis, and ligation. Human XPC-HR23B is thought to be the initial recognition factor in GGR and is required for recruiting transcription factor TFIIH to the damaged site (Sugasawa *et al.* 1998; Yokoi *et al.* 2000; Volker *et al.* 2001). XPB and XPD are subunits of the TFIIH complex that exhibit helicase activity of opposite polarity, which locally unwinds DNA around the lesion. Subsequently, the 3′ endonuclease XPG and XPA-RPA are loaded to the complex (Volker *et al.* 2001). XPA binds to DNA distortions, while RPA has a preference for single-stranded regions. XPA-RPA likely plays an architectural role in detecting bent DNA and in verifying whether the NER complex is correctly assembled on the damaged substrate before it is subjected to incision (Missura *et al.* 2001). After binding by XPF-ERCC1, incision occurs 3′ to the lesion by XPG and 5′ by XPF-ERCC1. In this way the damage is released in a 24- to 32-nucleotide-long oligonucleotide (Huang *et al.* 1992). DNA synthesis of the resulting gap requires polymerase δ or ε and the accessory factors RPA, PCNA, and RFC. Finally, the remaining nick is sealed by ligase I (de Laat *et al.* 1999; Petit and Sancar 1999). Human TCR is thought to be initiated by RNA polymerase II stalled at the damaged site (de Laat *et al.* 1999). All factors mentioned to be required for GGR, except XPC-HR23B, are also indispensable for TCR, while proteins that are specific for TCR are CSA and CSB.

Xeroderma pigmentosum (XP), representing the typical NER deficiency, is associated with extreme photosensitivity and development of skin cancer (de Boer and Hoeijmakers 2000). The XP phenotypes are likely caused by an accumulation of mutations, which are a consequence of inadequate removal of lesions or of error-prone translesion synthesis (Friedberg 2001). Consistently, a defect in POLH (also termed XPV or Pol η), which is involved in error-free damage bypass synthesis but not in NER, results in a XP-like phenotype (Masutani *et al.* 1999). In the absence of POLH, translesion synthesis is likely carried out by error-prone DNA polymerases.

Considerable progress in understanding the mechanism of NER has been also made with *Saccharomyces cerevisiae* (Thoma 1999; Prakash and Prakash 2000). An interesting difference in human NER is that Rad4-Rad23, the homologous factor of XPC-HR23B, functions in GGR and TCR (Verhage *et al.* 1994). Rad7 and Rad16, which do not have sequence homologues in humans, form a complex that acts in GGR (Verhage *et al.* 1994, 1996a). Rad26 of *S. cerevisiae*, like its homologue

[1]*Corresponding author:* Institute of Cell Biology, University of Bern, Baltzerstrasse 4, CH-3012 Bern, Switzerland.
E-mail: fleck@izb.unibe.ch

CSB, is involved in TCR but not in GGR (van Gool *et al.* 1994; Verhage *et al.* 1996a).

Sequencing of the genome of the fission yeast *Schizosaccharomyces pombe* has been recently completed (Wood *et al.* 2002), allowing identification of not-yet-characterized genes homologous to NER factors of other organisms. Interestingly, two open reading frames exist in *S. pombe*, whose deduced amino acid sequences show homology to human XPC and *S. cerevisiae* Rad4. This prompted us to analyze strains defective in one or both XPC homologues, named Rhp41 and Rhp42. We studied effects on cellular UV sensitivity, mitotic mutation avoidance, and meiotic mismatch repair. In addition, the relative contribution of Rhp41 and Rhp42 in GGR and TCR was addressed by a transcription recovery assay and by epistasis analysis, including *rhp7* (homologous to Rad7) and *rhp26* (homologous to Rad26 and CSB).

MATERIALS AND METHODS

Fission yeast media: *S. pombe* media of malt extract agar, YEA (yeast extract agar), and YEL (yeast extract liquid) were as described (Gutz *et al.* 1974). Minimal medium agar consists of 0.67% Difco nitrogen base without amino acids, 1% glucose, and 1.8% agar. PM (pombe minimal) and PM-N (PM without NH_4Cl) were described by Watanabe *et al.* (1988). Media were supplemented with adenine, histidine, leucine, lysine, and/or uracil (each 0.01%) where required. Concentrations of 0.01% G418 or 0.03% hygromycin B were included in YEA for selection and identification of *kanMX*- and *hphMX4*-based gene disruptions, respectively (see below).

***S. pombe* strains:** The *S. pombe* DNA repair mutants derived from SK15 (h^{90} *swi10::ura4 ura4-D18*; Rödel *et al.* 1992), OL455 (h^- *swi10::kanMX his3-D1 leu1-32 ura4-D18*; Kunz and Fleck 2001), J129 (h^- *uve1::LEU2 leu1-32 ura4-D18*; Yonemasu *et al.* 1997), Ru39 (h^- *msh2::his3 his3-D1*; Rudolph *et al.* 1999), TM1 (h^- *rhp42::kanMX ura4-D18*; this study), TM4 (h^+ *rhp41::ura4 ura4-D18 leu1-32*; this study), M4-230 (h^- *rhp7::kanMX his3-D1 leu1-32 ura4-D18*; this study), and TM106 (h^- *rhp26::hphMX4 his3-D1 ura4-D18*; this study). The checkpoint mutant *rad3::ura4 ura4-D18* has been described by Bentley *et al.* (1996). Construction of the *rev3::hphMX4* disruption strain will be reported elsewhere. The *ade6* alleles *485*, *M387*, and *51* were described by Schär and Kohli (1993) and *M210* and *M216* by Gutz (1963).

The gene disruption cassettes *rhp42::kanMX*, *rhp7::kanMX*, and *rhp26::hphMX4* were obtained by PCR using two 120-nucleotide-long primers and either pFA6a-kanMX6 (Bähler *et al.* 1998) or pAG32 (Goldstein and McCusker 1999) as template. The *hphMX4* cassette confers cellular resistance to hygromycin B and was originally constructed to create an additional marker for PCR-mediated gene disruptions in *S. cerevisiae* (Goldstein and McCusker 1999). The successful construction of the *rhp26::hphMX4* strain revealed that the *hphMX4* resistance gene can be used for *S. pombe* as well. *S. pombe* strains were transformed with PCR products and plated on YEA. After overnight incubation at 30°, transformants were replica plated on YEA containing 0.01% G418 to select for *kanMX6*-based disruptions or on YEA containing 0.03% hygromycin B to select for *hphMX4*-based disruptions. Correct gene disruptions were identified by PCR using primers that annealed either 5′ or 3′ to the transformed cassettes and primers derived from the cassettes in reverse orientation.

A *rhp41::ura4* disruption cassette was obtained by fusion PCR with six primers, in which the *ura4* gene was fused with 478 bp of the 5′ flanking region of *rhp41* and with 472 bp of the 3′ flanking region of *rhp41*. Transformants were selected on minimal medium containing leucine and were characterized by PCR as described above.

Genetic tests and cytological procedures: Reversion rates of the *ade6* alleles *485* and *M387* were determined by fluctuation tests as described (Kunz and Fleck 2001). Meiotic mismatch repair was studied by intragenic two-factor crosses including closely linked mutations in the *ade6* gene (Schär and Kohli 1993; Rudolph *et al.* 1998). Intergenic recombination was studied with the cross *leu2-120* × *lys7-2* (Tornier *et al.* 2001). Tests for UV sensitivity of vegetative cells were performed as described (Kunz and Fleck 2001).

For meiotic time courses, cultures of diploid cells were grown in PM to a density of 5×10^6–1×10^7 cells/ml. Diploid cells were maintained by intragenic complementation of the *ade6* alleles *M210* and *M216* (Gutz *et al.* 1974). Meiosis was induced by a shift to the nitrogen-free medium PM-N. Proper meiosis was controlled by 4′,6-diamidino-2-phenylindole staining as described previously (Bähler *et al.* 1993). Completion of meiosis was monitored 24 hr after shift by formation of four-spored asci. To test survival of UV-irradiated diploid cells during meiosis, aliquots of cultures were taken immediately before shift (0 hr) to PM-N and every 2 hr thereafter. Aliquots were plated on YEA and irradiated with a UV dose of 20 J/m^2 in a UV Stratalinker (Stratagene, La Jolla, CA). After 5 days of incubation at 30°, survival was calculated from the number of cells that formed colonies relative to nonirradiated cells.

Recovery of RNA polymerase II synthesis after UV irradiation: RNA recovery assays were performed according to Reagan and Friedberg (1997). For RNA recovery in vegetative cells, transcription of *fbp1*, which is repressed by glucose, was measured (Vassarotti and Friesen 1985). Expression of *fbp1* is induced when cells are shifted from a medium containing 5% glucose to a medium containing 0.1% glucose. A 10-ml culture in YEL was grown overnight at 30° and used to inoculate 300 ml PM containing 5% glucose. The culture was shaken at 30° until a titer of 1–2×10^7 was reached. Cells were harvested by centrifugation, kept on ice, and resuspended in 300 ml H_2O. Three 100-ml aliquots were transferred to a plastic dish in such a way that the suspension was <2 mm in height. After irradiation in an UV Stratalinker (Stratagene), aliquots were pooled, harvested by centrifugation, and suspended in PM containing 0.1% glucose. A 50-ml aliquot was immediately frozen at −70° (time point 0). The remainder of the culture was incubated at 30° and 50-ml samples were taken at various time points afterward. To measure *fbp1* transcription of nonirradiated cells, 40-ml samples were taken.

For recovery of SPBC1289.14 RNA during meiosis, meiotic time courses were performed as described above. Six hours after induction of meiosis, cells were irradiated with 100 J/m^2 UV in a plastic dish as described above. Samples were taken immediately after irradiation and at various time points afterward.

Total RNA of the samples was isolated as described (Grimm *et al.* 1991). After electrophoresis, RNA was transferred to a GeneScreen Plus nylon membrane (New England Nuclear) using a vacuum blotter (Amersham Pharmacia Biotech). Hybridization was performed with radiolabeled PCR fragments of *fbp1* or of SPBC1289.14 as described previously (Schweingruber *et al.* 1992).

RESULTS

Identification of two XPC homologues in *S. pombe*: Two open reading frames that encode homologues of Rad4 of *S. cerevisiae* and XPC of human and other species have been identified in *S. pombe*. After consultation with

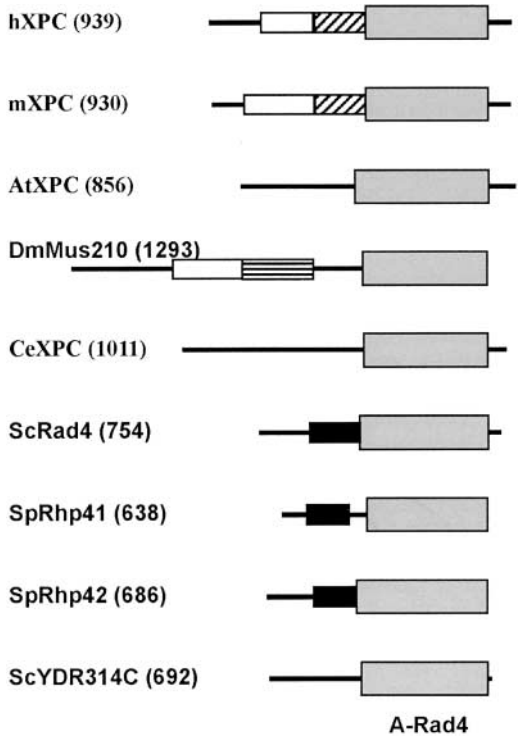

FIGURE 1.—Members of the XPC family. Proteins are from human (hXPC), mouse (mXPC), *Arabidopsis thaliana* (AtXPC), *D. melanogaster* (DmMus210), *Caenorhabditis elegans* (CeXPC), *S. cerevisiae* (ScRad4 and ScYDR314C), and *S. pombe* (SpRhp41 and SpRhp42). The number of amino acids in the proteins is given in parentheses. All XPC homologues share a common protein family A domain (BATEMAN *et al.* 2002) in their C-terminal regions (large gray boxes marked A-Rad4). Other regions are conserved only between some of the homologues (open, solid, or diagonally or horizontally striped small boxes). Data were obtained from the Internet (http://www.sanger.ac.uk/Software/Pfam/).

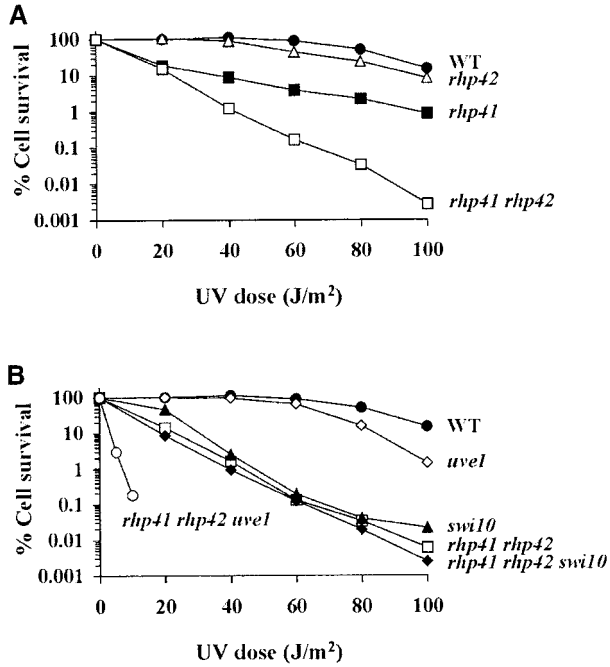

FIGURE 2.—Rhp41 and Rhp42 are involved in NER of UV-induced damages. (A) UV sensitivity of *rhp41* and *rhp42* mutants. (B) Epistasis analysis with the NER mutant *swi10* and the UVER mutant *uve1*. Data are mean values from three independent experiments.

the *S. pombe* gene-naming committee (http://www.genedb.org/genedb/pombe/geneRegistry.jsp), we named the homologues Rhp41 and Rhp42 (*R*ad *h*omologue *p*ombe *4-1* and *4-2*). However, it should be noted that the names Rhp4A and Rhp4B have been recently used as synonyms for Rhp41 and Rhp42, respectively (FUKUMOTO *et al.* 2002).

Interestingly, in addition to Rad4, a second protein with similarity to XPC also exists in *S. cerevisiae* (Figure 1). Rhp41 and Rhp42 are more similar to each other (37% identity in 602 amino acids) than to *S. cerevisiae* Rad4 (33% identity in 600 amino acids and 28% in 675 amino acids, respectively) or to YDR314C (28% identity in 657 amino acids and 25% identity in 692 amino acids, respectively). Homology of the four yeast proteins to XPC of multicellular eukaryotes is rather limited to the C-terminal region. In this region, human XPC is 35% identical to Rhp41 and Rhp42 (in 354 and 361 amino acids, respectively), 30% to Rad4 (in 380 amino acids), and 28% to YDR314C (in 217 amino acids).

Rhp41 and Rhp42 act in the NER pathway: We first addressed the question of whether Rhp41 and Rhp42 have a function in repair of damages induced by UV irradiation and, if so, whether they are involved in NER. Therefore, *rhp41* and *rhp42* gene disruption mutants were created and tested for UV sensitivity together with *swi10* and *uve1* mutant strains. The *swi10* gene encodes a homologue of human ERCC1 and causes a total NER defect (RÖDEL *et al.* 1992; FLECK *et al.* 1999; HOHL *et al.* 2001). The *uve1* gene encodes the 5′ endonuclease Uve1, which is essential for the NER-independent UV-damage-repair (UVER) pathway (YONEMASU *et al.* 1997; MCCREADY *et al.* 2000). When exposed to UV, the *rhp42* mutant turned out to be only slightly more affected than a wild-type strain, while the *rhp41* mutant showed moderate sensitivity (Figure 2A). Cell survival was further reduced in the *rhp41 rhp42* double mutant and was in the range of a *swi10* single mutant and of a *rhp41 rhp42 swi10* triple mutant (Figure 2B). In contrast, a *rhp41 rhp42 uve1* mutant was clearly more sensitive to UV radiation. Consistent with a previous study (FUKUMOTO *et al.* 2002), our data demonstrate that Rhp41 and Rhp42 are components of NER but not of the UVER pathway and that Rhp41 is more important than Rhp42 for UV damage repair in vegetative cells.

Transcription is blocked in UV-irradiated *rhp41* cells: To specifically analyze repair of damages in the transcribed strand, we measured transcription of the inducible *fbp1* gene after cellular exposure to UV (see MATERIALS AND METHODS). The presence of UV-induced damage in the transcribed strand blocks transcription, while removal of such damage allows recovery of tran-

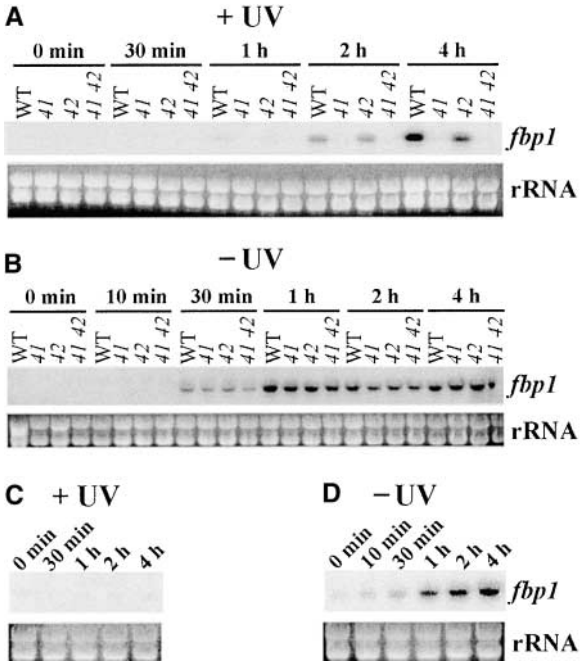

FIGURE 3.—Transcription of *fbp1* in UV-irradiated and nonirradiated NER mutant cells. (Top) *fbp1* RNA detected by Northern hybridization. (Bottom) Ethidium-bromide-stained rRNA of the same samples, used as loading controls. Aliquots of cultures were taken for RNA preparation at the indicated time points after induction of *fbp1*. (A) Transcription is blocked by UV damage in *rhp41* and *rhp41 rhp42* cells. Cultures were irradiated with 100 J/m^2 UV immediately before induction. Strains were wild type (WT), *rhp41* (*41*), *rhp42* (*42*), and *rhp41 rhp42* (*41 42*), all additionally mutated in *uve1*. (B) Transcription of *fbp1* is not significantly affected in nonirradiated cells. The same strains were used as in A. (C) Block of transcription in UV-irradiated *rhp41* cells is not a consequence of checkpoint activation. A culture of the *rad3 rhp41 uve1* strain was irradiated with 100 J/m^2 UV immediately before *fbp1* induction. (D) Transcription of *fbp1* is not significantly affected in nonirradiated *rad3 rhp41 uve1* cells.

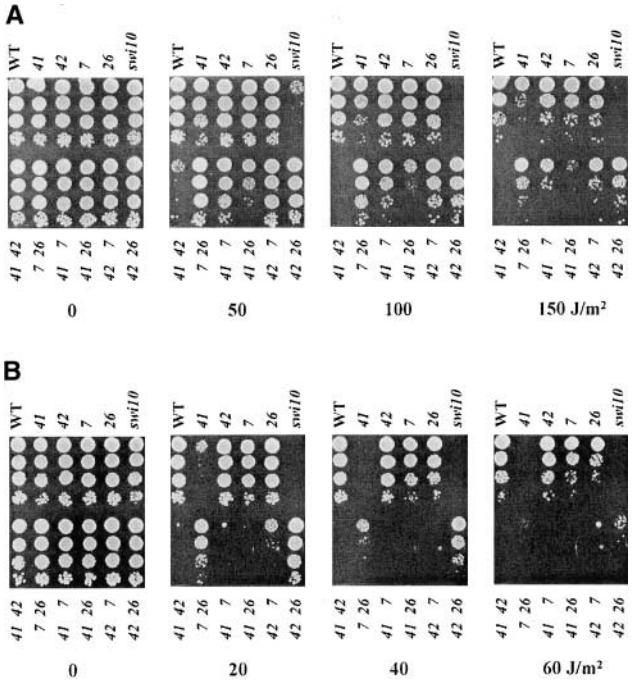

FIGURE 4.—Epistasis analysis for UV sensitivity between *rhp41*, *rhp42*, *rhp7*, and *rhp26* mutants in *uve1*$^+$ (A) and *uve1*Δ (B) background. Serial dilutions were dropped on full medium, irradiated with the indicated UV doses (in joules per square meter), and incubated for 3 days at 30°. WT, wild type; *41*, *rhp41*; *42*, *rhp42*; *7*, *rhp7*; and *26*, *rhp26*.

scription. To prevent interference with repair mediated by UVER, all strains were deleted for *uve1*. In cells proficient for NER or mutated in *rhp42*, *fbp1* mRNA was detectable at a low level 1 hr after irradiation and in increasing amounts after 2 and 4 hr (Figure 3A). In contrast, transcription was almost completely blocked in *rhp41* and *rhp41 rhp42* mutants; even after 4 hr, only traces of *fbp1* mRNA could be detected.

In a control experiment, *fbp1* expression of nonirradiated cells was measured. No obvious difference could be observed between the strains (Figure 3B). Thus, transcription of *fbp1* was not significantly affected by inactivation of *rhp41* and *rhp42*. To ensure that block of transcription in *rhp41*-deficient cells was not due to damage-induced checkpoint activation, we tested *fbp1* expression in *rad3* background, which causes a defect in DNA damage and replication checkpoint pathways (CASPARI and CARR 1999). Transcription of *fbp1* was severely blocked when *rad3 rhp41 uve1* cells were exposed to UV (Figure 3C), but was not affected when the same strain was not irradiated (Figure 3D). Together, these data show that block of transcription in UV-irradiated *rhp41* cells is not due to altered transcription caused by mutated *rhp41* or due to *rad3*-dependent checkpoint activation. Therefore, Rhp41 is important for repair of UV-induced damages in the transcribed strand and thus for TCR.

Epistasis analysis on UV survival, including *rhp7* and *rhp26*: The existence of two XPC homologues in *S. pombe* suggests that Rhp41 and Rhp42 have distinct roles in the NER mechanism. One possibility is that one protein acts in TCR and the other in GGR. To test this, *rhp41* and *rhp42* mutations were combined with disrupted *rhp7*, causing a defect in GGR (LOMBAERTS *et al.* 1999), and with *rhp26*, partially affected in TCR (YASUHIRA *et al.* 1999). In the following, epistasis analysis on UV sensitivity (Figure 4), mitotic mutation avoidance (Table 1), and meiotic mismatch repair (Table 2) was performed. The results are summarized in Table 4.

Survival of UV-treated cells was studied in *uve1*$^+$ (Figure 4A) and *uve1*Δ (Figure 4B) background. In *uve1*$^+$ background, *rhp41* showed reduced cell survival after UV irradiation, while *rhp26*, *rhp7*, and *rhp42* were as resistant as wild type (Figure 4A). *rhp41 rhp26*, but not *rhp41 rhp7*, was more sensitive than *rhp41* to UV. On the other hand, *rhp42 rhp7*, but not *rhp42 rhp26*, was more sensitive than either single mutant (Figure 4A). These data indicate that in the presence of functional UVER, Rhp41 contributes to GGR and Rhp42 to TCR.

In *uve1*Δ background, survival of UV-irradiated cells

TABLE 1

Mitotic mutation rates

Genotype	*485* to Ade$^+$		*M387* to Ade$^+$	
	Rate[a]	Increase[a]	Rate	Increase
Wild type	4.0 (1.3)	1.0	2.9 (0.7)	1.0
msh2	52 (14)	13	ND	—
swi10	29 (9.7)	7.3	9.5 (0.6)	3.3
rhp41	11 (2.3)	2.8	5.6 (2.9)	1.9
rhp42	3.7 (3.3)	0.9	2.9 (1.2)	1.0
rhp41 rhp42	40 (6.0)	10	9.3 (1.5)	3.2
msh2 rhp41	94 (27)	24	ND	—
msh2 rhp42	34 (5.8)	8.5	ND	—
msh2 rhp41 rhp42	109 (34)	27	ND	—
swi10 rev3	5.8 (2.1)	1.5	2.3 (0.9)	0.8
rhp41 rhp42 rev3	2.6 (1.0)	0.7	2.2 (1.3)	0.8
rhp7	1.1 (1.2)	0.3	ND	—
rhp26	2.7 (0.9)	0.7	ND	—
rhp7 rhp26	39 (8.7)	10	ND	—
rhp41 rhp7	26 (7.0)	6.5	ND	—
rhp41 rhp26	45 (11)	11	ND	—
rhp42 rhp7	1.2 (1.0)	0.3	ND	—
rhp42 rhp26	2.6 (4.4)	0.7	ND	—

Numbers are mean values of Ade$^+$ revertants per 10^9 cell divisions with standard deviations in parentheses. Reversion rates were determined from at least three independent experiments. ND, not determined.

[a] Relative to wild type.

TABLE 2

Prototroph frequencies in intragenic two-factor crosses

Genotype	*485* × *M387*	*485* × *51*	*M216* × *51*
Wild type	167 (16)	14 (1.5)	2108 (618)
msh2	144 (50)	116 (27)	ND
swi10	9.3 (2.4)	5.3 (1.0)	1954 (148)
rhp41	138 (53)	10 (1.8)	5035 (2188)
rhp42	34 (6.7)	4.2 (1.0)	1979 (766)
rhp41 rhp42	10 (1.2)	3.2 (0.8)	5879 (2194)
msh2 rhp41	146 (74)	118 (59)	ND
msh2 rhp42	29 (14)	29 (2.3)	ND
msh2 rhp41 rhp42	12 (3.3)	12 (0.7)	ND
swi10 rev3	8.1 (0.4)	ND	ND
rhp41 rhp42 rev3	12 (1.3)	ND	ND
rhp7	164 (12)	17 (4.4)	ND
rhp26	186 (39)	17 (4.6)	ND
rhp7 rhp26	170 (23)	16 (1.5)	ND
rhp41 rhp7	129 (21)	10 (1.3)	ND
rhp41 rhp26	185 (33)	9.9 (2.0)	ND
rhp42 rhp7	8.7 (2.3)	3.1 (1.0)	ND
rhp42 rhp26	16 (1.6)	2.9 (0.4)	ND

Numbers are mean values of prototrophic recombinants per 10^6 colony-forming spores with standard deviations in parentheses. Crosses were performed at least three times. Cross *485* × *M387* is illustrated in Figure 5. ND, not determined.

was strongly reduced for *rhp41*, slightly affected in *rhp7* and *rhp26*, and not different from wild type with the *rhp42* mutant strain (Figure 4B). All NER double mutants showed reduced survival when compared to respective NER single mutants.

Role of the NER factors in mitotic mutation avoidance: Increased mutation rates have been previously reported for mutants of *S. pombe*, which have a total defect in NER (Fleck *et al.* 1999). We were therefore interested to know how mutation rates were altered in the *rhp41*, *rhp42*, *rhp7*, and *rhp26* mutants, which appeared to be only partially affected in NER of UV-induced damages. We measured reversion rates of the *ade6* alleles *485* (a C-to-G transversion) and *M387* (a G-to-C transversion).

The *485* reversion rate of the *rhp42* mutant was as low as in wild type, slightly higher for *rhp41*, and as strongly increased in the *rhp41 rhp42* double mutant as in *swi10* (Table 1). The same tendency could be observed with the *M387* allele. Compared to the mismatch repair (MMR) mutant *msh2*, *485* reversion rates were further increased in *msh2 rhp41* and *msh2 rhp41 rhp42* mutants, but not in *msh2 rhp42* mutants (Table 1). Thus, Rhp41 is more important than Rhp42 for mitotic mutation avoidance.

Previous studies revealed that increased mutation rates in NER mutants of *S. cerevisiae* are dependent on functional DNA polymerase Z, which is implicated in error-prone translesion synthesis (Roche *et al.* 1994; Harfe and Jinks-Robertson 2000). PolZ was originally termed Pol ζ; the new name is according to the revised nomenclature for DNA polymerases (Burgers *et al.* 2001). To test whether PolZ is responsible for increased reversion rates of the base-substitution alleles *485* and *M387* in NER-deficient strains of *S. pombe*, we inactivated *rev3*, which encodes the catalytic subunit of PolZ (Broomfield *et al.* 2001). Indeed, rates were lowered to the wild-type level in the *swi10 rev3* and *rhp41 rhp42 rev3* strains (Table 1). Thus, *485* and *M387* reversions in NER mutants are primarily due to error-prone DNA synthesis by PolZ.

The reversion rate of *rhp26* was in the range of wild type, while that of *rhp7* was even lower (Table 1). However, the *rhp7 rhp26* double mutant exhibited a mutator phenotype similar to *rhp41 rhp42*, indicating that NER-dependent mutation avoidance is completely inactivated when *rhp7* and *rhp26* are both mutated. A similar rate was found with the *rhp41 rhp26* strain and a somewhat lower rate with the *rhp41 rhp7* strain. The *rhp42 rhp26* double mutant showed about the same level of revertants as wild type, while *rhp42 rhp7*, like *rhp7*, showed an even lower rate.

Rhp42 plays a major role in short-patch mismatch correction during meiosis: A sensitive assay to study repair of mismatches produced during meiotic recombination is based on the formation of prototrophic recombinants that arise in intragenic two-factor crosses of strains with two closely linked mutations in the *ade6* gene (Schär and Kohli 1993). When mutated sites are included in the recombination intermediate, two defined mismatches are formed in the same hetero-

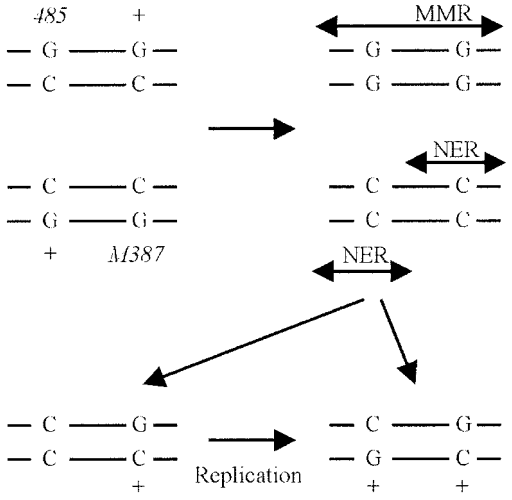

FIGURE 5.—Formation and repair of mismatches produced in the intragenic two-factor cross *485* × *M387*. When both mutated sites are included in heteroduplex DNA during recombination, two G/G mismatches are formed in one chromatid and two C/C mismatches are formed in the second chromatid. The G/G mismatches are frequently corepaired by the long-patch MMR pathway, which prevents formation of prototrophs. The C/C mismatches are not substrate of MMR and can be independently repaired by NER. Prototrophs arise when one mismatch is repaired toward wild-type information and subsequent replication (left) or when both C/C mismatches are repaired toward wild-type information (right).

duplex DNA (Figure 5). Corepair of both mismatches in the same strand omits production of Ade$^+$ cells, since one of the mismatches is repaired toward the mutant allele. Ade$^+$ can arise when both mismatched sites are converted toward wild-type information. This occurs when nucleotides are independently corrected on opposite strands or when one mismatch is repaired toward wild-type information, while the second remains unrepaired. In this case, a prototrophic cell is produced after subsequent replication (Figure 5). In both cases, short-patch repair of mismatches is required for prototroph formation. Our previous studies revealed that inactivation of NER results in a drop of prototroph frequencies, because short-patch repair is affected, accompanied by more frequent long-patch repair by the MMR system (Fleck *et al.* 1999; Kunz and Fleck 2001).

In this study, we tested the effects of the NER mutants *rhp41*, *rhp42*, *rhp7*, and *rhp26* on prototroph frequencies of the two-factor crosses *ade6-485* × *ade6-M387* and *ade6-485* × *ade6-51* (Table 2). In *485* × *M387*, two C/C or two G/G mismatches separated by 25 bases can arise in the same chromatid (Figure 5). In *485* × *51*, a C/C and a T/G mismatch, separated by 21 bases, can be formed in one chromatid, and a G/G and a C/A mismatch can be formed in the second chromatid. Compared to wild-type crosses, frequencies were slightly reduced in *rhp41*, significantly reduced in *rhp42*, and strongly reduced in *rhp41 rhp42* crosses. Mutated *msh2* caused an increased prototroph frequency in the cross *485* × *51*, but not in the cross *485* × *M387*, as expected from previous studies (Rudolph *et al.* 1998; Fleck *et al.* 1999; Kunz and Fleck 2001). Similar results were obtained with crosses including *msh2 rhp41* double mutants, while *msh2 rhp42* gave significantly lower frequencies. In addition, crosses with the *msh2 rhp41 rhp42* triple mutant showed a further decrease. These data revealed that Rhp41 and Rhp42 have a function in MMR-independent meiotic mismatch repair and that Rhp42 is more important. NER-dependent repair of mismatches produced during meiotic recombination is not influenced by PolZ, since additional disruption of *rev3* in *swi10* and *rhp41 rhp42* crosses did not alter prototroph frequencies (Table 2).

TABLE 3

Intergenic recombination in the cross *lys7* × *leu2*

Genotype	% recombinants	Increase[a]
Wild type	11.1 (2.0)	1.0
swi10	13.4 (1.3)	1.2
rhp41	9.3 (5.3)	0.8
rhp42	9.4 (1.2)	0.8
rhp41 rhp42	12.7 (4.7)	1.1

Random spores were analyzed for recombination beween *lys7* and *leu2*. Numbers are mean values of at least three experiments with standard deviations in parentheses.

[a] Relative to wild type.

The occurrence of Ade$^+$ in *rhp7*, *rhp26*, and *rhp7 rhp26* backgrounds was not different in wild type (Table 2). In addition, frequencies of *rhp41 rhp7* and *rhp41 rhp26* crosses were about in the range of *rhp41*. On the other hand, *rhp42 rhp26* and *rhp42 rhp7* produced fewer prototrophs than *rhp42* produced in the cross *485* × *M387*, but not in the cross *485* × *51*.

To test whether reduction of prototroph frequencies is indeed caused by a defect in short-patch mismatch repair or whether it reflects a defect in recombination, we performed the crosses *ade6-M216* × *ade6-51* and *leu2* × *lys7*. Since mutated sites in the intragenic cross *M216* × *51* are separated by 1219 bp, most prototrophic recombinants are formed independently of short-patch mismatch repair. The *rhp42* cross gave a frequency similar to wild type, while a 2.4-fold increase was observed with *rhp41* and a 2.9-fold increase with *rhp41 rhp42* (Table 2). The *rhp41*-dependent increase appeared to be not due to a defect in NER, since the *swi10* mutant behaved like wild type. None of the NER mutants showed a significantly altered recombination frequency in the intergenic cross *leu2* × *lys7* (Table 3). Thus, low prototroph frequencies in intragenic two-factor crosses with closely linked mutations are not due to a general recombination defect of NER mutants.

The *rhp41* mutant is affected in UV damage repair during meiosis: Our analysis revealed that the *rhp41* mutant has a major defect in damage repair in vegetative cells, while inactivation of *rhp42* affects mainly short-

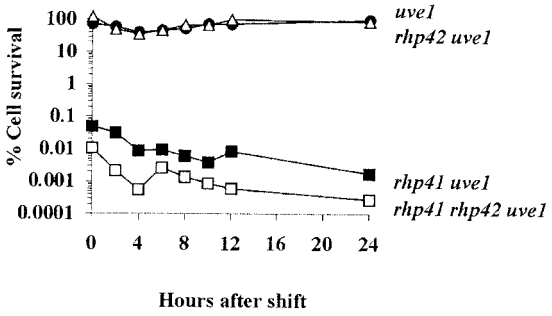

FIGURE 6.—The *rhp41* mutant is sensitive to UV during meiosis. Aliquots of cultures were taken at the indicated time points after induction of meiosis, plated on full medium, irradiated with 20 J/m^2 UV, and incubated for 5 days at 30°. Data are mean values from two experiments.

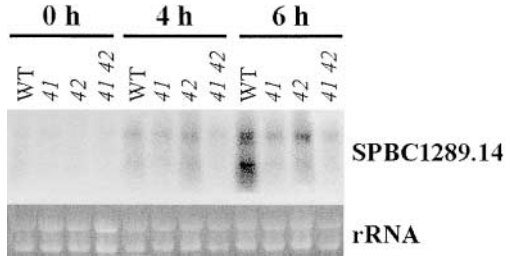

FIGURE 7.—Transcription of SPBC1289.14 during meiosis is blocked by UV damage in the *rhp41* mutant. Six hours after induction of meiosis, cells were exposed to UV (100 J/m^2). (Top) SPBC1289.14 RNA of cells taken at the indicated time points after UV irradiation was detected by Northern hybridization. (Bottom) Ethidium-bromide-stained rRNA as loading control. Diploid strains, all additionally mutated in *uve1*, were wild type (WT), *rhp41* (*41*), *rhp42* (*42*), and *rhp41 rhp42* (*41 42*).

patch mismatch correction during meiosis. We therefore studied how repair of damages produced during meiosis is affected in the *rhp41* and *rhp42* mutants. For this analysis, all strains were additionally deleted for *uve1*. The *rhp42* strain was as resistant to UV as the NER-proficient strain during all stages of meiosis, while *rhp41* and *rhp41 rhp42* were clearly more sensitive (Figure 6).

We next tested meiotic cells for transcription of the SPBC1289.14 gene after exposure to UV (Figure 7). SPBC1289.14, encoding a putative class II aldolase involved in carbohydrate metabolism (http://www.sanger.ac.uk/), is upregulated 8–12 hr after induction of meiosis (http://www.sanger.ac.uk/PostGenomics/S_pombe/projects/sexualdifferentiation/). We identified two transcripts of different size. In cells collected immediately after UV irradiation, weak expression of SPBC1289.14, which represents constitutive transcription that could not be blocked by damage, was detected (Figure 7). The level of both types of RNA increased 4 and 6 hr after UV treatment. After 6 hr, the amount of RNA, especially in the smaller species, was less in *rhp42* cells than in wild-type cells. Importantly, block of transcription was stronger in *rhp41* cells (Figure 7).

DISCUSSION

To learn more about the role of XPC and homologous proteins, we analyzed the *S. pombe rhp41* and *rhp42* mutants with respect to their defects in DNA repair. Epistasis analysis placed both to NER and revealed that Rhp41 is more important for repair of damaged DNA, while Rhp42 is involved in short-patch mismatch repair. However, the *rhp41 rhp42* double mutant was in all cases more affected than either single mutant. Thus, repair defects caused by inactivation of one gene can be partially compensated by the function of the second gene.

Role of Rhp41 and Rhp42 in GGR and TCR: The status of TCR in UV-irradiated cells was tested by RNA recovery assays. Transcription was strongly blocked in *rhp41* cells, but not or only slightly affected in *rhp42* cells. However, the data obtained from the epistasis analyses with *rhp7* and *rhp26* indicate that Rhp41 and Rhp42 act in both GGR and TCR (see also Table 4). In *uve1*Δ background, additional inactivation of *rhp7* or *rhp26* caused further UV-induced cell killing. The *rhp41 rhp7* and *rhp41 rhp26* mutants had higher *485* reversion rates than *rhp41* had, and *rhp42 rhp7* and *rhp42 rhp26* gave lower prototroph frequencies in the cross *485* × *M387* than *rhp42* gave. On the other hand, repair defects were generally stronger in *rhp41 rhp26* than in *rhp41 rhp7* mutants and were more pronounced in *rhp42 rhp7* than in *rhp42 rhp26* strains. Most strikingly, in *uve1*$^+$ background the *rhp41 rhp7* double mutant behaved like *rhp41* when exposed to UV. The observation that the *rhp41 rhp26* mutant showed stronger defects in damage repair than *rhp41 rhp7* showed can be explained in two ways. First, GGR is more impaired than TCR in *rhp41* mutant cells. Second, in addition to a defect in GGR, *rhp41* causes inactivation of Rad26-dependent and -independent TCR. Our analysis further revealed that the *rhp42 rhp7* mutant exhibited stronger repair defects than *rhp42 rhp26* exhibited. Since mutated *rhp7* results in a total defect in GGR (LOMBAERTS *et al.* 1999), additional effects caused by *rhp42* likely reflect a defect in TCR. Again, the finding that the *rhp42 rhp26* mutant is more sensitive than the *rhp26* mutant to UV in *uve1*Δ background may be due to an additional defect in GGR caused by loss of *rhp42* or due to inactivation of Rhp26-independent TCR.

The contribution of the two XPC homologues of *S. pombe* in repair of UV-induced damages has also been analyzed in a recent study (FUKUMOTO *et al.* 2002). The study revealed that Rhp41 has a major function in removal of cyclobutane pyrimidine dimers (CPDs) in the transcribed and the nontranscribed strands of the *rbp2* gene, which is consistent with our data. However, in contrast to our results, Rhp42 appeared to act solely in repair of the nontranscribed strands and thus in GGR. This difference is likely due to the different approaches used in the two studies. The repair assay by FUKUMOTO

TABLE 4

Summary of epistasis analyses

Genotype	Vegetative cells: UV sensitivity[a] *uve1*$^+$	Vegetative cells: UV sensitivity[a] *uve1*Δ	Vegetative cells: Mutation rate[b]	Meiotic cells: Prototroph frequency[c]
rhp7 rhp26	S	S	S	WT
rhp41 rhp7	E	S	S	E
rhp41 rhp26	S	S	S	E
rhp42 rhp7	S	S	WT[d]	S
rhp42 rhp26	WT	S	WT	S
rhp41 rhp42	S	S	S	S

Repair defects of double mutants are expressed as S, E, or WT. S (synergistic or additive effect): defect stronger than that of either single mutant. E (epistatic relationship): defect not different from that of the single mutant with the stronger defect. WT (wild type): not different from wild type.

[a] Original data on UV sensitivity of vegetative cells are shown in Figure 4.

[b] Mitotic mutation rates are listed in Table 1.

[c] Prototroph frequencies are given in Table 2.

[d] Mutation rate lower than that in wild type.

et al. (2002) allowed detection of CPD removal from both strands of a gene directly, while with the RNA recovery assay repair of damages in the transcribed strand was measured rather indirectly. On the other hand, lesions other than CPDs, most importantly 6-4 pyrimidine pyrimidone photoproducts (6-4PPs), can be detected with the RNA recovery assay. Both assays do not allow detection of minor effects on repair, which led Fukumoto *et al.* (2002) to the conclusion that Rhp42 has no function in TCR. The same conclusion may be drawn considering only the data on RNA recovery (Figures 3 and 7). However, the epistasis analysis with *rhp7* and *rhp26* enabled detection of minor effects on TCR and GGR. The finding that the *rhp42 rhp7* double mutant is clearly more sensitive than either single mutant to UV therefore suggests that Rhp42 has a function in TCR. Since the conclusion that Rhp7 is indispensable for GGR is based on strand-specific repair of CPDs (Lombaerts *et al.* 1999), an alternative explanation of the data is that, in the course of GGR of UV-induced damage, Rhp7 is exclusively involved in removal of CPDs and Rhp42 in repair of 6-4PPs. This possibility should be addressed in future experiments.

Several studies revealed that human GGR is initiated by XPC-HR23B, which binds to lesions and subsequently recruits TFIIH (Sugasawa *et al.* 1998; Yokoi *et al.* 2000; Volker *et al.* 2001), while human TCR does not require the function of XPC-HR23B. Instead, TCR is thought to be initiated by RNA Pol II stalled at a lesion, which in a subsequent step requires CSA and CSB for release of the transcription machinery (de Boer and Hoeijmakers 2000). A remarkable difference in human XPC is that *S. cerevisiae* Rad4 and *S. pombe* Rhp41/Rhp42 are involved in TCR (Verhage *et al.* 1994; Fukumoto *et al.* 2002; this study). These data suggest that either TCR is initiated by the yeast XPC homologues, rather than by a stalled RNA Pol II complex, or XPC proteins are not the initial damage recognition factor. The latter possibility is consistent with a recent study on human NER, which showed that the RPA subunit p70 binds to a psoralen crosslink before XPC is assembled (Reardon and Sancar 2002).

Human XPC and *S. cerevisiae* Rad4 form a tight complex with HR23B and Rad23, respectively (Prakash and Prakash 2000; Friedberg 2001). With Rhp23, a single ortholog has also been identified in *S. pombe* (Wood *et al.* 2002). Mutated *rhp23* causes moderate UV sensitivity and is like the *rad23* mutant of *S. cerevisiae* affected in TCR and GGR (Mueller and Smerdon 1996; Lombaerts *et al.* 2000). This is consistent with the findings that Rad4 of *S. cerevisiae* and Rhp41 and Rhp42 of *S. pombe* are involved in both NER subpathways (Verhage *et al.* 1994; this study).

Increased mutation rates in NER mutants originate from PolZ-dependent translesion synthesis: It has been previously shown in *S. cerevisiae* that vegetative cells defective in NER exhibit increased frameshift reversion rates (Harfe and Jinks-Robertson 2000). In contrast to wild type, where mainly 1-bp insertions occurred, NER mutants accumulated complex events in which 1-bp insertions were flanked by one or more base substitutions in the vicinity (Harfe and Jinks-Robertson 2000). Increased rates and the occurrence of complex mutations were dependent on Rev1 and PolZ, which catalyze mutagenic bypass of lesions (Friedberg and Gerlach 1999). Rev1 predominantly inserts dCMP opposite a variety of damages, producing a replication intermediate that can be extended by PolZ (Nelson *et al.* 1996; Zhang *et al.* 2002). However, recent work revealed that Rev1 can also insert dGMP and TMP opposite undamaged guanines and apurinic/apyrimidinic sites (Masuda *et al.* 2002).

In this study, we have shown that increased reversion rates of base substitutions in NER mutants of *S. pombe* are also dependent on PolZ. Spontaneous mutation rates were not different between *rhp42* and wild type, were slightly higher in *rhp41*, and further increased in *rhp41 rhp42*. Thus, as for repair of UV damages, Rhp41 contributes more than Rhp42 does to mutation avoidance. The mutator phenotype of NER mutants may reflect PolZ-dependent synthesis across undamaged, damaged, or mismatched DNA. Error-prone replication of undamaged DNA is unlikely to cause the mutator, since a defect in NER rather reflects the failure to process damaged or, eventually, mismatched DNA. The data obtained with intragenic two-factor crosses revealed that NER is able to correct mismatches during meiotic recombination (Fleck *et al.* 1999). It is therefore conceivable that vegetative cells deficient in NER accumulate mutations as a direct consequence of unrepaired mis-

matches. In this case, the mutator phenotype could be due to mismatch extension by PolZ. However, this possibility is attractive only when NER-dependent mismatch processing is different in vegetative cells and during meiosis, since mutation rates were not increased in the *rhp42* mutant, which otherwise exhibited a moderate effect on mismatch correction during recombination. Thus, it is more likely that spontaneous lesions account for increased mutation rates in NER mutants.

Our previous studies revealed that the base substitution *485* predominantly reverted by G:C-to-C:G transversions in NER mutants (Fleck *et al.* 1999; Kunz and Fleck 2001). PolZ-dependent accumulation of G:C-to-C:G transversions in principle can originate from misincorporation of a guanine opposite a damaged or a lost guanine present in the nontranscribed strand or of a cytosine opposite a damaged or a lost cytosine in the transcribed strand. G:C-to-C:G changes then occur by subsequent replication or by repair of the damage. The observation that the *rhp7 rhp26* double mutant showed a high reversion rate suggests that reversions can originate from damages in both strands and thus that more than one type of lesion accounts for error-prone bypass synthesis.

NER-dependent mismatch repair during meiotic recombination: Since the mutator phenotype of NER mutants is dependent on functional PolZ, error-prone bypass synthesis of endogenous lesions, rather than the failure to repair mismatches, is responsible for increased mutation rates in vegetative cells. However, several lines of evidence suggest that mismatches are a direct substrate of NER, at least during meiotic recombination. First, prototroph frequencies in intragenic two-factor crosses are strongly dependent on the types and on the distance of the mismatches that can be formed in heteroduplex DNA (Schär and Kohli 1993; Fleck *et al.* 1999). Second, NER-dependent short-patch repair of mismatches produced during meiosis is not dependent on PolZ (this study). Third, *swi10*-dependent increase of postmeiotic segregation (PMS) of the *ade6* allele *16C* in a monofactorial cross in *pms1 swi10* background is in the range of 5%, which is orders of magnitude higher than the occurrence of any spontaneous lesion (Fleck *et al.* 1999). Thus, it is most likely that NER of *S. pombe* is able to process mismatched bases directly. A limited set of data indicates that mismatches can be also corrected by NER of other species. A defect of *mei-9* (homologous to human XPF) of *Drosophila melanogaster* causes increased PMS frequencies *in vivo* and affects repair of mismatches *in vitro* (Carpenter 1982; Bhui-Kaur *et al.* 1998). In addition, NER of *Escherichia coli* and humans is able to incise mismatched substrates *in vitro* (Petit and Sancar 1999).

Our present study revealed a major contribution of Rhp42 in NER-dependent meiotic mismatch repair in *S. pombe*. The apparently preferential function of Rhp42 during meiosis is not mirrored by a transcriptional induction. Transcription levels of *S. pombe* genes during meiosis were determined recently using DNA microarrays (Mata *et al.* 2002; http://www.sanger.ac.uk/PostGenomics/S_pombe/projects/sexualdifferentiation/). Transcription of *rhp42* was not significantly altered in wild-type cells during vegetative growth and at different stages of meiosis. On the other hand, a threefold induction of *rhp41* expression could be detected between 5 and 8 hr after induction of meiosis, at which time S phase and the two meiotic divisions take place. Furthermore, transcription of both genes, *rhp41* and *rhp42*, in vegetative cells is about two- to threefold induced in response to UV irradiation (Fukumoto *et al.* 2002).

Why two XPC homologues in yeast? Rhp41 and Rhp42 exhibit about the same degree of homology to other proteins of the XPC family, which does not allow speculating about their relative contribution in NER. YDR314C, the second XPC homologue of *S. cerevisiae*, has not yet been characterized. However, sensitivity of the *rad4* mutant to UV radiation, nitrogen mustard, and methyl methanesulfonate is as pronounced as for other NER mutants, suggesting that Rad4 is indispensable for NER of damages (Gietz and Prakash 1988; Xiao and Chow 1998; McHugh *et al.* 1999). Consistently, repair of UV damage is abolished in both strands of RNA Pol II-transcribed genes and in the genome overall (Verhage *et al.* 1994). One notable exception is that removal of UV damage in the transcribed strand of RNA Pol I-transcribed rDNA genes occurs independently of Rad4 (Verhage *et al.* 1996b).

Initiation of TCR by RNA Pol II stalled at a lesion presupposes that in the presence of many damages, transcription has to be newly initiated after a first round of repair and that it is again blocked at the next lesion downstream, a process that appears to be time and energy consuming. Therefore, multicellular organisms may prefer to undergo apoptosis of severely damaged cells. In contrast, survival is the better choice for unicellular organisms, which therefore may attempt to repair also heavily damaged DNA. This may be easily achieved when yeast XPC homologues are also implicated in TCR, since multiple sites of damage in the same gene can be simultaneously repaired. In addition, TCR might be more important in *S. pombe* and *S. cerevisiae*, since these organisms have compact genomes. In *S. pombe*, ~58% of the genome represents coding sequences (Wood *et al.* 2002).

The existence of two XPC homologues in yeast may allow extension of the substrate spectrum of NER. In fact, while Rhp41 is preferentially directed to repair damaged DNA, Rhp42 has a function in meiotic mismatch repair. Short-patch repair of mismatches during recombination allows fast diversification of genomes, which might be important for a quick response to environmental changes as well as for evolution in general.

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