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The *RAD7* and *RAD16* Genes, Which Are Essential for Pyrimidine Dimer Removal from the Silent Mating Type Loci, Are Also Required for Repair of the Nontranscribed Strand of an Active Gene in *Saccharomyces cerevisiae*

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The *rad16* mutant of *Saccharomyces cerevisiae* was previously shown to be impaired in removal of UV-induced pyrimidine dimers from the silent mating-type loci (D. D. Bang, R. A. Verhage, N. Goosen, J. Brouwer, and P. van de Putte, Nucleic Acids Res. 20:3925–3931, 1992). Here we show that *rad7* as well as *rad7 rad16* double mutants have the same repair phenotype, indicating that the *RAD7* and *RAD16* gene products might operate in the same nucleotide excision repair subpathway. Dimer removal from the genome overall is essentially incomplete in these mutants, leaving about 20 to 30% of the DNA unrepaired. Repair analysis of the transcribed *RPB2* gene shows that the nontranscribed strand is not repaired at all in *rad7* and *rad16* mutants, whereas the transcribed strand is repaired in these mutants at a fast rate similar to that in *RAD*$^+$ cells. When the results obtained with the *RPB2* gene can be generalized, the RAD7 and RAD16 proteins not only are essential for repair of silenced regions but also function in repair of nontranscribed strands of active genes in *S. cerevisiae*. The phenotype of *rad7* and *rad16* mutants closely resembles that of human xeroderma pigmentosum complementation group C (XP-C) cells, suggesting that *RAD7* and *RAD16* in *S. cerevisiae* function in the same pathway as the XPC gene in human cells. RAD4, which on the basis of sequence homology has been proposed to be the yeast XPC counterpart, seems to be involved in repair of both inactive and active yeast DNA, challenging the hypothesis that RAD4 and XPC are functional homologs.

DNA damage can be repaired with various efficiencies for different parts of the genome. For UV-induced cyclobutane pyrimidine dimers, this differential repair has been described for organisms ranging from *Escherichia coli* to humans (3, 5, 13, 17, 18, 33, 34, 38, 39). Efficient repair of transcribed strands of active genes is thought to contribute to increased survival of the cells as a result of fast recovery of RNA synthesis that is otherwise blocked by UV damage. In *E. coli*, a factor that is coupling repair to transcription, transcription repair coupling factor (TRCF), has been found to be essential for the preferential repair of the transcribed strand (29, 30). Such a coupling factor might be missing in human Cockayne's syndrome (CS) cells, since in these cells the transcribed strand is repaired at the rate of the genome overall, accompanied by an increase of UV sensitivity of these cells (43, 45). The repair defect in CS-B cells is complemented by the *ERCC6* gene (40, 41), suggesting that ERCC6 might be the eukaryotic counterpart of the *E. coli* TRCF. However, in contrast to human CS-B mutants, *E. coli mfd* mutants that are missing the TRCF are hardly UV sensitive, suggesting that in *E. coli*, transcription-coupled repair does not lead to increased survival after UV irradiation. The reversed situation exists in rodent cells and in human XPC cell lines that remove dimers in only a relatively small part of the genome. In these cells, transcribed strands of active DNA are repaired efficiently, whereas dimers in the remainder of the genome are not removed, which results in only a modest UV sensitivity of these cell types (3, 12, 18, 46, 47). Preferential repair of the transcribed strand has been shown to exist in the yeast *Saccharomyces cerevisiae* for the *URA3* gene on a minichromosome (33), for the chromosomal as well as episomal *RPB2* gene (34), and for the transcriptionally induced *GAL7* gene (13). In the latter two cases, strand-specific repair was shown to be dependent on a functional RNA polymerase II (13, 34).

The active *MAT*α locus in *S. cerevisiae* is repaired faster than its silent copy *HML*α irrespective of transcription (36), a result that might be explained by the difference in chromatin structure between these otherwise identical loci. The *MAT*α locus has an open structure, while the inactive *HML*α locus is silenced by a complex of several factors that impose a heterochromatin-like structure upon this locus (21). This silenced locus therefore might need extra factors that make it accessible for the repair enzymes, while the *MAT* locus can be repaired directly because of its open structure. A generally accepted model for differential repair discriminates between two possibilities: first, heterochromatin-like structures can cause a delay in repair; second, repair can be coupled to transcription, causing preferential repair of transcribed strands (5, 13, 34–36, 43, 44). In *S. cerevisiae*, mutants in the *RAD3* epistasis group are disturbed in nucleotide excision repair (8, 25). Two members of this group, *rad7* and *rad16*, are only moderately UV sensitive, which is suggestive of an accessory role of the affected gene products in excision repair. However, we have previously shown that the *RAD16* gene product is essential for repair of the *HML*α locus (2). One possible explanation might be that RAD16 functions in accessibility of closed heterochromatin-like structures (2). The *RAD16* gene has been cloned, and its open reading frame (ORF) contains putative helicase

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TABLE 1. *S. cerevisiae* strains used in this study

Strain	Genotype	Source[a]
LP2741-3B	*MATα ade1 his3-1 leu2-3 leu2-112 trp1 ura3-52 rad7-1*	L. Prakash
W303	*MATα ho ade2 trp1 leu2 can1 his3 ura3*	R. Rothstein
W303236	*rad16Δ::URA3*[b]	Constructed
MGSC97	*rad7Δ::URA3*[b]	Constructed
MGSC104	*rad7Δ::LEU2*[b]	Constructed
MGSC105	*rad7Δ::LEU2 rad16Δ::URA3*[b]	Constructed
MG70/X9B-7B	*MATα gal ade2-1 rad4-4*	YGSC[c]

[a] The strains were gifts of the investigators mentioned or constructed as described in the text.
[b] The remainder of the genotype conforms to that of W303.
[c] YGSC, Yeast Genetic Stock Centre.

motifs that are present in a wide variety of eukaryotic proteins, one of which is SNF2, a transcription factor that might interact with chromatin components to overcome repressive effects of chromatin (24, 49). Here we show that *rad7* mutants have the same phenotype as *rad16* mutants and further elucidate the role of the *RAD7* and *RAD16* genes in excision repair. Our results indicate a role for the *RAD7* and *RAD16* gene products in repair of silenced regions of the genome and of nontranscribed strands of active DNA. The repair phenotype of *rad7* and *rad16* mutants resembles that of a human xeroderma pigmentosum complementation group C (XP-C) mutant. XP-C is a rare human autosomal recessive disorder that is characterized by a variety of clinical symptoms. The moderate UV sensitivity of XP-C cells was shown to be resulting from a deficiency in repair of nontranscribed DNA, whereas transcribed DNA is normally repaired (46, 47). *rad7* and *rad16* cells have a repair phenotype similar to that of XP-C mutants, but RAD7, RAD16, and XPC are not homologous on the protein sequence level. We determined whether the *rad4* mutant of *S. cerevisiae* has the same repair phenotype as *rad7*, *rad16*, or XP-C cells, since *RAD4* has been proposed as the yeast homolog of the XPC gene on the basis of limited protein sequence homology (10, 14). We show that repair of both the transcribed and nontranscribed strand of an active gene is absent in a *rad4* mutant, indicating that RAD4 is, unlike XPC, RAD7, and RAD16, necessary for repair of inactive as well as transcribed DNA, a result that challenges the presumption that RAD4 is the yeast homolog of XPC.

MATERIALS AND METHODS

Strains and growth conditions. The yeast strains used in this study are listed in Table 1. Cells were grown in complete medium (YEPD; 1% yeast extract, 2% Bacto Peptone, 2% glucose) at 28°C under vigorous shaking conditions.

Plasmids and bacterial strains. All plasmids were cloned by standard molecular biology techniques (15). Plasmids were propagated in *E. coli* JM101 or DH5α under appropriate antibiotic selection.

Yeast transformations. Yeast transformations were carried out by electroporation (2,250 V/cm, 250 μF, 200 Ω). Cells were plated on YNB (0.67% yeast nitrogen base, 2% glucose, 2% Bacto Agar) with appropriate amino acids and incubated at 28°C for 2 to 5 days. Transformants were restreaked several times on selection medium before they were used in subsequent experiments.

***rad16* disruption mutant.** In plasmid pUB23, the *Hin*dIII fragment of *RAD16* is replaced by *URA3* as described previously (2). This plasmid was linearized with *Pvu*I and subsequently transformed to strain W303 by electroporation to obtain strain W303236.

***rad7* disruption mutants.** In plasmid pDG79 (a kind gift of R. D. Gietz), the *Xho*I-*Hin*dIII fragment in the *RAD7* ORF on a 4-kb *Eco*RI chromosomal fragment in pBR322 is replaced by *URA3*. This plasmid was linearized with *Eco*RI and transformed to W303 to obtain strain MGSC97.

To obtain disruptions of *rad7* with the *LEU2* gene as a selectable marker, the 4-kb *Eco*RI *RAD7* fragment of pGP5 (gift of G. Perozzi; pGP5 is the same as pGP4 [23] but with the *Eco*RI fragment in the opposite direction) was cloned in pIC20R to obtain pIC20R-RAD7. An *Eco*RI-*Hin*dIII *LEU2* fragment from plasmid pDG317 (gift of R. D. Gietz; the *Eco*RI and *Kpn*I restriction sites from the *LEU2* gene are destroyed) was cloned in pUC21 to obtain pUC21-LEU2. The *Kpn*I-*Hin*dIII fragment of the *RAD7* ORF in pIC20R-RAD7 was replaced by a *Kpn*I-*Hin*dIII *LEU2* fragment from pUC21-LEU2. The resulting construct, pRAD7Δ::LEU2, was linearized with *Bgl*I and transformed to W303 to obtain strain MGSC104 and to strain W303236 to obtain strain MGSC105.

In all cases, successful disruption was confirmed by Southern blotting.

UV irradiation and DNA isolation. Yeast cells diluted in chilled phosphate-buffered saline were irradiated with 254 nm of UV light (Philips T UV 30W) at a rate of 3.5 J/m^2/s. Subsequently, cells were collected by centrifugation, resuspended in growth medium, and incubated for various times in the dark at 28°C prior to DNA isolation as described by Sherman et al. (32). DNA was purified on CsCl gradients (15).

T4 endoV isolation. T4 endonuclease V (endoV) was isolated from *E. coli* cells containing a plasmid with the *denV* gene, which can be induced by isopropylthiogalactopyranoside (IPTG) (gift of J. K. de Riel). The enzyme was purified as described by Nakabeppu et al. (20) to a 95% purity, as shown by silver-stained gels.

Specific probes. (i) α. From plasmid pAK5 (gift of A. Klar), which contains the *Xho*I fragment of *HMLα*, we subcloned a *Sca*I fragment containing parts of X, Yα, and Z1. Subsequently, a *Dra*I fragment, which is internal in Yα and strictly α specific, was inserted into pUC19. From this plasmid, an *Eco*RI-*Hin*dIII fragment containing the α-specific DNA was isolated.

(ii) a. From plasmid pA164 (gift of M. Hall), which contains the *Eco*RI-*Hin*dIII fragment of *HMR***a**, a 250-bp Y**a** internal *Ssp*I-*Bgl*II fragment was inserted in the *Bam*HI-*Hin*cII sites of pUC12. From this plasmid, an *Eco*RI-*Hin*dIII fragment containing the **a**-specific DNA was isolated.

(iii) *RPB2*. The oligonucleotides 5′GGTATTATTCCAGA CGGTG and 5′GTTAAAAACACACCCATAGC were synthesized, and PCR was performed on yeast chromosomal DNA with these primers (35 cycles, annealing temperature of 35°C), which yielded a 1.4-kb fragment. The PCR product was digested with *Eco*RI and *Xho*I, and the resulting 1-kb fragment was cloned in M13 (*Eco*RI-*Sal*I) in both orientations for isolation of single-stranded probes. The PCR product was used as a double-stranded *RPB2* probe. All double-stranded DNA probes were labeled by using random hexanucleotides and [α-^{32}P]dCTP.

(iv) Single-stranded M13-derived probes. Single-stranded M13 with the cloned gene of interest was isolated as described by Maniatis et al. (15). Subsequently, primer extension was performed (42) from an M13 hybridization primer that points outward from the polylinker. Therefore, M13 but not the cloned insert was used as a template for the primer extension. Incorporation of [α-^{32}P]dCTP led to labeled probes that recognized only one strand of the *RPB2* gene.

Primer extension reactions were carried out by mixing ca. 500 ng of single-stranded template and 4 pmol of M13 hybridization primer in 10 μl with final concentrations of 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, and 100 mM NaCl. This mixture was boiled for 3 min and incubated at 37°C for 30 min. Then 4 μl of a mix of 1 mM dGTP, dATP, and dTTP was added along with 2 μl of Boehringer buffer L, 2 μl of water, 1 μl of [α-^{32}P]dCTP, and 1 μl of Klenow enzyme (1 U/μl). The reaction mix was incubated at room temperature for 1 h, and unincorporated nucleotides were removed by gel filtration in a G-50 column. The labeled probe was used directly (without denaturation) for hybridization.

Southern blot analysis showing the removal of endonuclease-sensitive sites (ESS). (i) *MATα/HMLα* repair. Genomic DNA was cut with restriction endonuclease *Hae*II, which generates a 3.6-kb *MATα* fragment and a 4.0-kb *HMLα* fragment. DNA samples were then divided in two equal parts, one of which was incubated with T4 endoV, and both were loaded on denaturing agarose gels as described by Bohr et al. (3). After electrophoresis, the DNA was transferred to Hybond-N+ (Amersham) and hybridized with the α-specific probe.

(ii) *HMRa* repair. To check whether *HMRa* was repaired in an α haploid strain, genomic DNA was cut with *Hin*dIII to generate a 4.9-kb *HMRa* fragment. The rest of the procedure was as described above except that an **a**-specific probe was used.

(iii) *RPB2* repair. The procedure described above was used except that genomic DNA was cut with *Pvu*I and *Pvu*II, which gives a 5.2-kb *RPB2* fragment (34), and *RPB2*-specific probes were used. After hybridization of the probe for the first strand, the probe was removed from the blot by alkaline washing buffer, and the same blot was subsequently used for hybridization with the probe that detected the opposite strand.

The amount of hybridized labeled probe in each band on the Southern blots was quantified with a Betascope 603 blot analyzer (Betagen) and used to calculate the amount of dimers per fragment with the following equation: amount of dimers per fragment = −ln (counts in T4 endoV-treated lane/counts in nontreated lane). For all samples, this value was divided by the value obtained at $t = 0$, subtracted from 1, and multiplied by 100 to give the percentage of repair. After being scanned in the blot analyzer, autoradiographs were prepared from the Southern blots.

Determination of overall repair by using antibodies. Repair of the genome overall was determined as described in detail by Roza et al. (26). In short, 100 ng of denatured genomic yeast DNA was absorbed per well of poly-L-lysine-precoated plates in duplicate or in triplicate. With these plates, an enzyme-linked immunosorbent assay (ELISA) was performed (7) with monoclonal antibodies raised against GT<>TG oligonucleotides that specifically recognize T<>T dimers and do not cross-react with other UV-induced lesions, e.g., 6-4 photoproducts (H3 antibodies [26]). Dose-response experiments showed that fluorescence increases linearly as a function of the applied UV dose in the range in which the experiments were performed (result not shown). Fluorescence was recorded with a Fluoroskan (Flow Laboratories). Alternatively, a slot blot immunoassay was used (48). Briefly, 100 ng of heat-denatured DNA was slot blotted onto nitrocellulose (0.1-μm pore size; Schleicher & Schuell). After being baked for 1 h at 80°C under vacuum, the filters were incubated with H3 antibodies overnight at 4°C. Subsequently, the filters were washed and incubated with rabbit anti-mouse horseradish peroxidase for 2 h and washed again. Peroxidase activity resulted in emission of chemiluminescent light after addition of luminol. This light was detected on photographic films (exposure for 2 to 20 s), and the films were quantified by scanning with a densitometer (Ultroscan XL; Pharmacia LKB).

RESULTS

***rad7* mutants are impaired in DNA repair of the silent mating-type loci.** After UV irradiation of *S. cerevisiae* cells, the expressed *MATα* locus is repaired faster than its silenced copy *HMLα* (39). These loci are identical in sequence and differ only in transcriptional activity and chromatin structure. The heterochromatin-like structure of the *HMLα* locus might need extra factors to make it accessible for the repair enzymes. We showed that the *RAD16* gene is required for repair of *HMLα* (2), suggesting that *RAD16* might be such an accessibility factor (2). We have also shown that a *rad7* mutant strain was unable to repair *HMLα* after UV irradiation (37). However, the strain that was used in that study (LP2741-3B; gift of L. Prakash) contains a deletion in its DNA that extends further than the *RAD7* gene (23, 31). Plasmid pGP5 containing the *RAD7* gene (gift of G. Perozzi) complemented the UV sensitivity as well as the inability to repair *HMLα* of strain LP2741-3B (results not shown). To obtain definite proof for the involvement of *RAD7* in repair of the silent mating-type loci, we constructed a *rad7* disruption mutant in which most of the ORF of this gene was replaced by a *URA3* cassette. We then analyzed repair of the mating-type loci in the *rad7* disruption mutant. The Southern blot in Fig. 1B confirms that the *rad7* mutant is deficient in repair of the silent *HMLα* locus, whereas the *MATα* locus is repaired in this strain, although less efficiently than in *RAD*$^+$ cells. The other silent mating-type locus, *HMRa*, is also not repaired in a *rad7* strain (result not shown). These results indicate that both *RAD16* and *RAD7* are essential for repair of the silent mating-type loci.

A *rad7 rad16* double mutant has the same phenotype as either single mutant. Since *rad7* disruption mutants have the same phenotype for repair of *HMLα* as *rad16* disruption mutants, both *RAD7* and *RAD16* might function in one step of a pathway by forming a complex or function separately by performing subsequent steps in this pathway. Alternatively, there might be two pathways for repair of *HMLα*, one in which *RAD7* is involved and one dependent on *RAD16*. To investigate possible interactions between *RAD7* and *RAD16*, a double-disruption mutant was constructed by replacing more than two-thirds of the *RAD7* ORF by a *LEU2* cassette in strains that already contained a disruption of the *RAD16* gene. As expected, this double mutant was deficient in repair of *HMLα* (Fig. 1C). Survival as a function of the UV dose was measured (Fig. 2). No difference in survival is observed for both the *rad7* and *rad16* mutant and the double mutant, which are less UV sensitive than a *rad2* mutant (Fig. 2), suggesting that *RAD7* and *RAD16* are involved in the same nucleotide excision repair subpathway.

Repair of the genome overall is incomplete in *rad7* and *rad16* mutants. To find out whether the *RAD7* and *RAD16* genes have a function in dimer removal only from heterochromatin-like regions or also from other regions of the genome, we determined the fraction of the genome that is not repaired in *rad7* or *rad16* mutants. For this determination, we made use of monoclonal antibodies directed against cyclobutane pyrimidine dimers (26). DNA isolated after different repair periods after UV irradiation was incubated with these antibodies and subjected to an ELISA. The signal in this ELISA is a measure for the relative amount of pyrimidine dimers in the genome. Using this method, we compared the removal of dimers in *rad7* and *rad16* mutants and in *RAD*$^+$ cells. As can be seen in Fig.

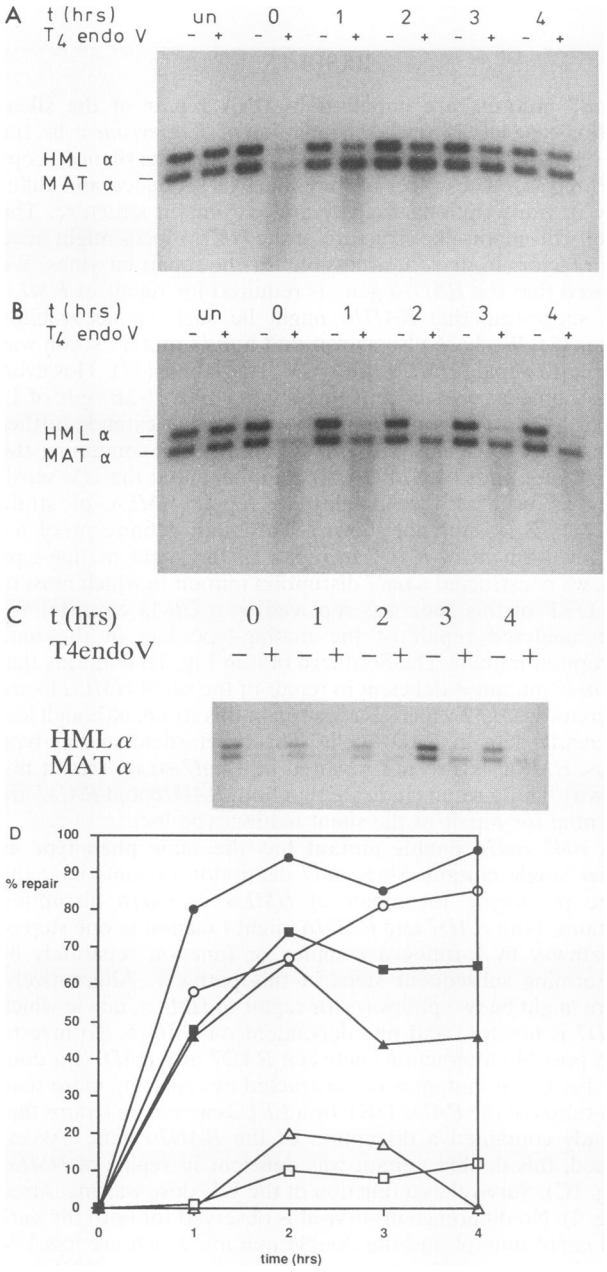

FIG. 1. Southern blots showing the removal of ESS from *MAT*α and *HML*α. Time points are indicated. un, DNA from an unirradiated control sample. Samples were mock treated (−) or treated with T4 endoV (+). The upper 4-kb fragment is *HML*α, and the lower 3.6-kb fragment is *MAT*α. (A) Autoradiograph of strain W303 (*RAD*$^+$); (B) autoradiograph of strain MGSC97 (*rad7*); (C) autoradiograph of strain MGSC105 (*rad7 rad16*); (D) percentage of dimer removal as a function of time. Blots shown in panels A to C were quantified with a Betascope blot analyzer, percent repair was calculated as described in Materials and Methods. Symbols in panel D: ●, *RAD*$^+$ *MAT*α; ○, *RAD*$^+$ *HML*α; ■, *rad7 MAT*α; □, *rad7 HML*α; ▲, *rad7 rad16 MAT*α; △, *rad7 rad16 HML*α. Repair of *HML*α is absent in the *rad7* and *rad7 rad16* strains, whereas *MAT*α is repaired in this mutant, albeit less efficiently than in W303.

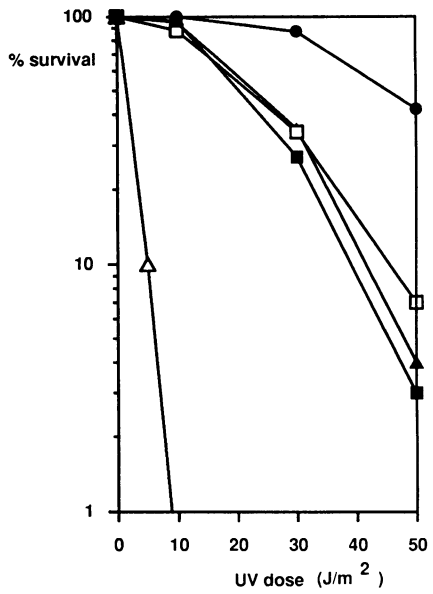

FIG. 2. UV survival curves for isogenic *RAD*$^+$, *rad7*, *rad16*, and *rad7 rad16* double mutants. Cells were grown in YEPD to an optical density of 0.6, diluted in water, and irradiated with the indicated UV doses, and dilutions were plated on YEPD. After 3 days of incubation at 28°C in the dark, colonies were counted and survival was calculated. Strain W303 (*RAD*$^+$) is rather insensitive to UV (circles), while strains W303236 (*rad16*; open squares), MGSC104 (*rad7*; filled squares), and MGSC (*rad7 rad16*; filled triangles) are equally UV sensitive. To indicate the intermediate UV sensitivity of *rad7* and *rad16* mutants, the survival curve of a *rad2* strain is also depicted (open triangles).

3, removal of dimers from a *RAD*$^+$ strain is almost completed within 2 h after irradiation, whereas in isogenic *rad7* and *rad16* mutants, part of the genome is refractory to repair, also when the repair period is prolonged. The results indicate that *RAD7* and *RAD16* are essential for repair of about 20 to 30% of the yeast genome. Regions in the yeast genome that could be heterochromatin-like apart from the silent mating-type loci might include the telomeres (1, 9) and part of the silenced rDNA cluster; these regions constitute too small a fraction (11) to account for the 20 to 30% that is not repaired in *rad7* or *rad16* mutants. It therefore seems that RAD7 and RAD16 are not solely accessibility factors for heterochromatin-like regions during excision repair. This observation is also consistent with the finding that the active *MAT*α locus is repaired less efficiently in *rad7* and *rad16* mutants than in *RAD*$^+$ cells (Fig. 1 and references 2 and 37).

***RAD7* and *RAD16* are essential for repair of the nontranscribed strand of *RPB2*.** In human XP-C cell lines, only a subfraction of the genome is repaired (12), and this repair is confined to transcribed strands of active genes in these cells (46, 47). As shown above, the part of the genome that is not repaired in *rad7* and *rad16* mutants is likely to consist of more than only the silenced regions. We investigated whether this nonrepaired fraction, by analogy to XP-C, includes also nontranscribed strands of active genes. For this purpose, we analyzed repair of both individual strands of a transcribed gene that was previously shown to be repaired strand specifically, i.e., the *RPB2* gene (34). We determined repair of *RPB2* in *RAD*$^+$ cells and in *rad7* and *rad16* disruption mutants. The Southern blots that were obtained when repair of the two strands of *RPB2* was analyzed in *rad7* and *rad16* mutants are shown in Fig. 4B and C. Clearly, in both mutants the nontran-

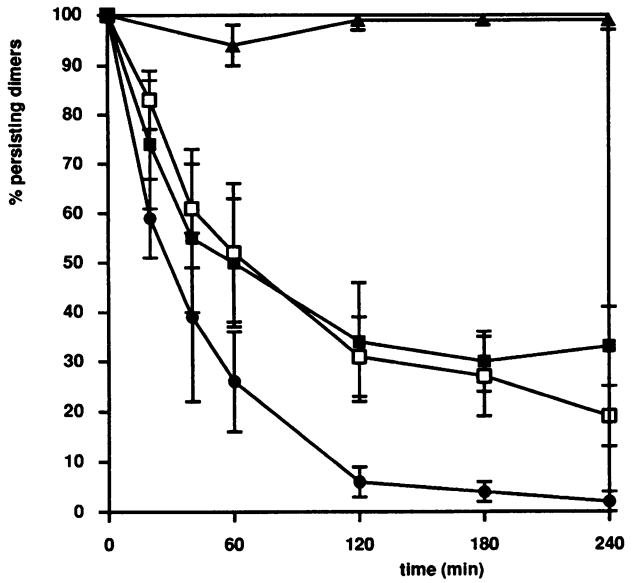

FIG. 3. Dimer removal from the genome overall as determined by an ELISA with antibodies directed against pyrimidine dimers. The percentage of persisting dimers as a function of time is shown; the initial amount was set at 100%. Dimer removal from W303 (*RAD*$^+$; circles) is very fast and complete, whereas dimer removal from the genome of MGSC97 (*rad7*; open squares) and W303236 (*rad16*; filled squares) is slower and essentially incomplete. Strain MG70/X9B-7B (*rad4*; triangles) does not remove dimers from its genome. Results of the ELISA are shown. Each point represents the average of two to five independent experiments; bars indicate standard errors. With a slot blot immunoassay (see Materials and Methods), similar results were obtained.

scribed strand of *RPB2* is not repaired at all, whereas the transcribed strand is repaired very efficiently, comparable to repair in *RAD*$^+$ cells (Fig. 4A). Identical results were obtained with the *rad7 rad16* double mutant (Fig. 4D). If the results obtained with *RPB2* are exemplary for other active yeast genes, the part of the yeast genome that is not repaired in *rad7* and *rad16* mutants consists not only of silenced regions but also of nontranscribed strands of active genes. This observation suggests that *RAD7* and *RAD16* may be essential for repair of nontranscribed DNA whereas DNA strands that are transcribed can be repaired independently of the *RAD7* and *RAD16* gene products.

Absence of dimer removal in a *rad4* mutant. The phenotype that we described above for *rad7* and *rad16* cells strongly resembles that of human XP-C cells, which are also intermediately UV sensitive as a consequence of a deficiency in repair of nontranscribed DNA but still able to repair transcribed strands of active DNA (46, 47). The sequence of the cloned XPC gene shows homology with the yeast *RAD4* gene on the protein level (14). Therefore, *RAD4* has been proposed to be the yeast homolog of the XPC gene. Since we find that *RAD7* and *RAD16* behave as functional homologs of XPC, we determined whether a *rad4* mutant has a phenotype similar to that of *rad7* and *rad16* mutants. When RAD4 and XPC are functionally homologous, it can be expected that the phenotype of *rad4* is the same as for *rad7* or *rad16* mutants. However, in contrast to the latter two intermediately UV sensitive mutants, *rad4* mutants are very UV sensitive (4, 8, 25). Moreover, it was reported previously that a *rad4* strain does not remove dimers from *MAT* and *HML* (37). In addition, we observe no dimer removal from the genome overall in a *rad4* strain (Fig. 3). Repair of *RPB2* in this *rad4* mutant was analyzed, and the resulting Southern blot is shown in Fig. 5. No repair of both individual strands of the *RPB2* gene is observed in this strain. These results suggest that *RAD4* is not involved in repair of only a subfraction of the yeast DNA and therefore may be not the functional yeast counterpart of XPC.

DISCUSSION

Mutations in the *RAD7* and *RAD16* excision repair genes confer only a moderate UV sensitivity to yeast cells. Previously we showed that *RAD16* is essential for repair of the silent *HML*α locus (2), and here we extend these findings by showing that *RAD7* is also essential for repair of this locus. Using antibodies directed against pyrimidine dimers, we show that a subfraction of the yeast genome is refractory to repair in *rad7* and *rad16* mutants. Strand-specific repair analysis reveals that dimers are not removed from the nontranscribed strand of the *RPB2* gene in these mutants. This finding suggests that the intermediate UV sensitivity of *rad7* and *rad16* strains is due to the inability to repair DNA that is not transcriptionally active, while transcribed strands of active DNA can be efficiently repaired in these mutants. The phenotype of *rad7* and *rad16* mutants resembles that of human XP-C cells, being proficient only in transcription-coupled repair (46, 47).

With an ELISA or with slot blotting using dimer-specific antibodies, we find that about 20 to 30% of the whole yeast genome is not repaired in *rad7* and *rad16* mutants after 4 h. Miller et al., using alkaline sucrose gradients detecting *Micrococcus luteus* nuclease-sensitive sites, report that about 46% of the DNA is not repaired in a *rad7* mutant (19). Apparently, only a limited fraction (20 to 50%) of the yeast genome is dependent on *RAD7* and *RAD16*. If the result for *RPB2* repair can be generalized to other yeast genes and RAD7 and RAD16 are involved in repair of all nontranscribed strands of active genes in yeast cells, the nontranscribed fraction of the genome seems to be rather low. Even when we take into account that the yeast genome has a very high information density (22) and a large part of genome is transcribed, the amount of nontranscribed DNA should in theory at least be 50%. However, part of the noncoding DNA that is not transcribed in the process of normal gene expression could be transcribed illegitimately occasionally or at a low level as a result of readthrough of transcription of other genes or to the presence of weak promoters. Such a low level of transcription could be sufficient to allow transcription-coupled repair and would then lower the percentage of DNA that needs the RAD7/RAD16 system for its repair. In this respect, it is of interest that we found genes in *S. cerevisiae* that have a repair profile different from that of *RPB2*. Notably, repair of the *PHO5-PHO3* locus in *RAD*$^+$ cells and in various excision repair mutants is suggestive of transcription on both the transcribed and nontranscribed strands, since both strands seem to be repaired by a transcription-coupled process independent of *RAD7* and *RAD16*, although we observe RNA from the transcribed but not from the nontranscribed strand on Northern (RNA) blots (unpublished results). Alternatively, *RAD7* and *RAD16* might not be essential for all nontranscribed sequences in the yeast genome.

RAD7 and *RAD16* not only function in repair of specific heterochromatin-like regions of the genome (2) but apparently are also involved in repair of dimers from nontranscribed strands in active DNA. It might very well be that the heterochromatin-like structure of *HML*α needs, besides RAD7 and RAD16, extra factors to make it accessible for the repair

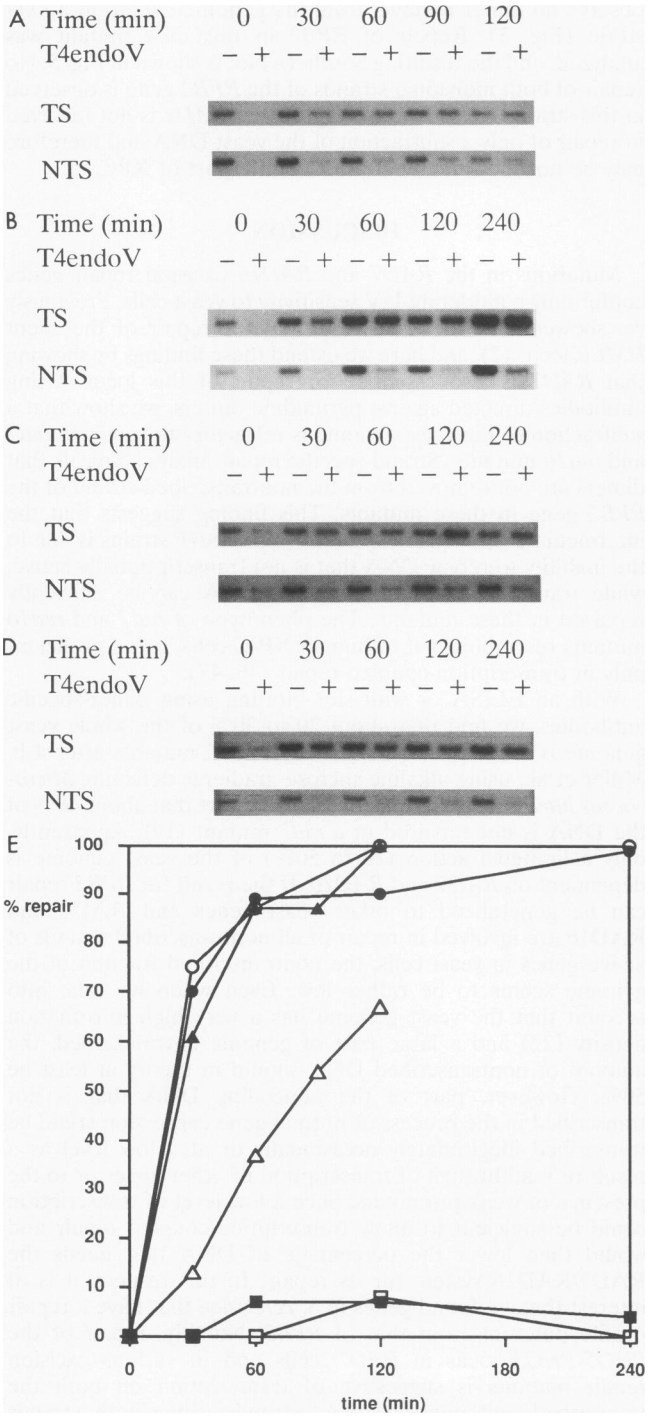

FIG. 4. Removal of ESS from both individual strands of the *RPB2* gene. Time points are indicated. Samples were mock treated (−) or treated with T4 endoV (+). Repair is analyzed in a 5.2-kb *Pvu*I-*Pvu*II fragment (34). TS, transcribed strand; NTS, nontranscribed strand. (A) Autoradiograph of strain W303 (*RAD*$^+$). (B) Autoradiograph of strain MGSC104 (*rad7*). (C) Autoradiograph of strain W303236 (*rad16*). (D) Autoradiograph of strain MGSC105 (*rad7 rad16*). The blots shown in panels A to C were quantified with a Betascope blot analyzer. For each time point, the amount of counts in the T4 endoV-treated lane was divided by the amount of counts in the mock-treated lane, and this value was used to calculate the percentage of repair in the fragment as described in Materials and Methods. A graphic representation of the obtained values is given in panel E. Symbols: ▲, *RAD*$^+$ TS; △, *RAD*$^+$ NTS; ●, *rad7* TS; ■, *rad7* NTS; ○, *rad16* TS; □, *rad16* NTS. Values slightly exceeding 100% because of loading discrepancies are depicted as 100%. In *rad7* and *rad16* cells, the TS is repaired at a fast rate similar to that observed in *RAD*$^+$ cells, whereas the NTS is not repaired at all.

enzymes, but this cannot be concluded from the data presented here and awaits further experimentation. The sequence of the *RAD16* ORF shows seven consecutive domains that constitute a putative helicase, a motif that has been found in various other eukaryotic proteins (2). One of these is SNF2, a transcription factor that was postulated to be involved in interacting with chromatin to allow transcription to take place in nucleosomal DNA (24, 49). The *RAD7* gene contains regions that are rather hydrophobic, and therefore the protein might be membrane bound (23), but further no clues about the function of this gene are found in its sequence. Whether RAD7 and RAD16 interact with chromatin or nucleosomes remains to be clarified.

RAD7 and *RAD16* are shown to be epistatic among the genes involved in nucleotide excision repair. Until now we have not found differences in phenotype for the two mutants or the double mutant, implying that the gene products might act together in a nucleotide excision repair subpathway. Possibly RAD7 and RAD16 form a complex; the protein sequence of RAD7 contains 12 tandemly repeated leucine-rich motifs that might be involved in protein-protein interactions (28). Whether RAD7 and RAD16 indeed interact with each other will be investigated by using the purified proteins. Since both *rad7* and *rad16* mutants in *S. cerevisiae* are only able to repair transcribed DNA analogous to human XP-C mutants (46, 47), RAD7 and RAD16 seem to have a function similar to that of XPC. However, no homology is found on the protein level between the *RAD7* or *RAD16* gene on one hand (2, 23) and the XPC gene on the other hand (14), not even when small domains are considered. The recent identification of interactions between the XPC protein and the human homolog of yeast RAD23, HHR23B (16), suggest that HHR23B mutations might lead to the same phenotype as displayed by XPC cells. However, humans who have mutations in HHR23B have not yet been identified, and by analogy, it may be possible that human *RAD7* or *RAD16* homologs exist, although patients with the phenotype to match have not been found. Yeast *rad23* mutants are not similar in phenotype to *rad7*, *rad16*, or XP-C mutants (our unpublished data).

The data that we present show that *RAD7* and *RAD16* behave functionally as homologs of XPC, whereas the *RAD4* gene was proposed to be the yeast homolog of XPC on the basis of the similarity between the protein sequences (4, 14). The homology between XPC and RAD4 is limited (14) but does not seem to be coincidental, since cloning of the *Drosophila* homolog of XPC revealed homology between the predicted polypeptide and that encoded by the yeast *RAD4* gene (10). However, *rad4* mutants, in contrast to XP-C mutants, are highly UV sensitive, and we found that repair of dimers from inactive as well as active DNA is completely absent in the *rad4-4* mutant that we tested. The *rad4-4* allele contains a −1 frameshift that leads to a complete loss of function (4). It remains possible that XPC has an extra function besides repair of nontranscribed DNA but that this function is not affected in the XPC cell lines that have been isolated. This would imply that a null mutation in XPC should be completely deficient in dimer removal, as in a *rad4* mutant. Another possibility could be that the *rad4* allele that we used in this study might be impaired in interaction with putative factors essential for

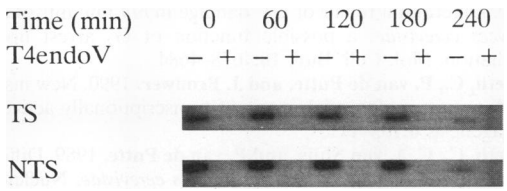

FIG. 5. Southern blot showing the removal of ESS from both strands of the *RPB2* gene in strain MG70/X9B-7B (*rad4-4*). Time points are indicated. Lanes containing samples mock treated or treated with T4 endoV are denoted by − or +, respectively. TS, transcribed strand; NTS, nontranscribed strand. The fragment analyzed is a 5.2-kb *Pvu*I-*Pvu*II fragment (34).

overall repair. These putative enzymes might then be unable to exert their function without RAD4, leading to complete absence of dimer removal. Alternative possibilities could also be envisaged.

The findings described in this report suggest that the nontranscribed strand is repaired by a different pathway than the transcribed strand. How do *RAD7* and *RAD16* function in repair of the nontranscribed strand? Probably the actual incision near the lesion and subsequent repair steps do not differ for the two strands. It is therefore likely that RAD7 and RAD16 are proteins that make the damage in the nontranscribed strand available for the repair enzymes. The transcribed strand of *RPB2* is repaired in *rad7* or *rad16* mutants at the same rate as in RAD^+ cells, suggesting that the gene products are not needed for excision repair of damage in the transcribed strand. Recognition of damage in active DNA is possibly provided for by the transcription machinery, while DNA that cannot be repaired by this mechanism is dependent on *RAD7* and *RAD16*. The presence of repair proteins in transcription factors in both human (27) and yeast (6) cells indicates a dual role for the transcription machinery, which might switch from transcription to repair when damage is encountered. Apparently, the transcription-dependent mode on the transcribed strand removes damage faster than the *RAD7*/*RAD16*-dependent step operating on the nontranscribed strand, resulting in preferential repair of transcribed strands. An important factor contributing to very efficient repair of transcribed strands might be ERCC6, since the *ERCC6* gene complements for the repair defect in CS-B cells (40, 41) that have lost the ability to perform strand-specific repair (43, 45). Recent findings suggest that the homolog of ERCC6 also in *S. cerevisiae* is involved in preferential repair of the transcribed strand (42). Experiments to investigate the relationship between the transcription-coupled and the *RAD7*/*RAD16*-dependent repair pathway are now in progress.

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