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Double Mutants of *Saccharomyces cerevisiae* with Alterations in Global Genome and Transcription-Coupled Repair

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The nucleotide excision repair (NER) pathway is thought to consist of two subpathways: transcription-coupled repair, limited to the transcribed strand of active genes, and global genome repair for nontranscribed DNA strands. Recently we cloned the *RAD26* gene, the *Saccharomyces cerevisiae* homolog of human *CSB*/*ERCC6*, a gene involved in transcription-coupled repair and the disorder Cockayne syndrome. This paper describes the analysis of yeast double mutants selectively affected in each NER subpathway. Although *rad26* disruption mutants are defective in transcription-coupled repair, they are not UV sensitive. However, double mutants of *RAD26* with the global genome repair determinants *RAD7* and *RAD16* appeared more UV sensitive than the single *rad7* or *rad16* mutants but not as sensitive as completely NER-deficient mutants. These findings unmask a role of *RAD26* and transcription-coupled repair in UV survival, indicate that transcription-coupled repair and global genome repair are partially overlapping, and provide evidence for a residual NER modality in the double mutants. Analysis of dimer removal from the active *RPB2* gene in the *rad7*/*16 rad26* double mutants revealed (i) a contribution of the global genome repair factors Rad7p and Rad16p to repair of the transcribed strand, confirming the partial overlap between both NER subpathways, and (ii) residual repair specifically of the transcribed strand. To investigate the transcription dependence of this repair activity, strand-specific repair of the inducible *GAL7* gene was investigated. The template strand of this gene was repaired only under induced conditions, pointing to a role for transcription in the residual repair in the double mutants and suggesting that transcription-coupled repair can to some extent operate independently from Rad26p. Our findings also indicate locus heterogeneity for the dependence of transcription-coupled repair on *RAD26*.

The molecular details of the versatile process of nucleotide excision repair (NER) are becoming increasingly clear as more of the proteins involved are purified and biochemically analyzed (1, 8, 17; for reviews about NER, see references 7 and 12). However, the process of differential repair, the difference in rate of removal of cyclobutane pyrimidine dimers from different parts of the genome (for reviews, see references 9 and 32), is not yet fully understood at the molecular level. Although the actual process of dimer removal is likely to be performed by the same repair enzymes in the same molecular way for the whole genome, one of the first steps of NER, DNA damage recognition in chromatin, might differ for lesions in specific regions. This notion has led to the idea of two subpathways of NER: (i) a process called global genome repair that is essential for removal of damage from nontranscribed DNA sequences and (ii) a system known as transcription-coupled repair that is involved in the specific fast and efficient repair of damage from the transcribed strand of active genes. Transcription-coupled repair has been found to occur in mammalian cells (16), *Escherichia coli* (15), and the yeast *Saccharomyces cerevisiae* (14, 27, 30), and it causes preferential repair of the transcribed strand over the nontranscribed strand and other inactive DNA.

Several genes have been shown to be specifically involved in each of the subpathways in mammals and in *S. cerevisiae*. In humans, the *XPC* gene is implicated in global genome repair, since cell lines with a mutation in this gene repair only dimers in transcribed strands of active genes (37). In *S. cerevisiae*, *rad7* or *rad16* disruption mutants are completely deficient in repair of the silent mating-type loci (2, 31) and the nontranscribed strand of an active gene (38). Repair of the transcribed strand is not affected in these mutants, suggesting that at least when the template strand is transcribed, the function of the global repair proteins Rad7p and Rad16p (and in human cells XPC) is restricted to repair of the nontranscribed strand.

Preferential repair of the transcribed strand over the nontranscribed strand was shown to be dependent on transcription (14, 30), indicating a role for the transcription process in efficient recognition of damage in transcribed DNA. In *E. coli*, a factor coupling the DNA repair machinery to transcription has been found (25). TRCF (transcription-repair coupling factor, the product of the *mfd* gene [24]) recognizes RNA polymerase stalled at a lesion, and through affinity for UvrA, it directs the repair enzymes to the damage; upon release of the polymerase-RNA complex, the lesion is repaired (25). Transcription-coupled repair has also been found to occur in higher eukaryotes, and it has been shown that this process requires transcription and additional proteins. One such protein is the *CSB* gene product, which complements the UV sensitivity and the deficiency in recovery of RNA synthesis after UV irradiation in Cockayne syndrome (CS) group B (CS-B) cells (33). CS-B cell lines have been shown to lack efficient repair of active DNA (35, 36), implying a role for the complementing gene *CSB* in transcription-coupled repair. We have cloned the yeast homolog of *CSB* and designated this gene *RAD26* (34). Disruption of this gene indeed leads to a defect in transcription-coupled repair of the *RPB2* gene in yeast cells (34). Remarkably,

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TABLE 1. *S. cerevisiae* strains used

Strain	Genotype	Reference
W303-1B	*MAT*α *ho can1-100 ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1*	21
W303236	*rad16*Δ::*URA3*[a]	38
MGSC97	*rad7*Δ::*URA3*[a]	38
MGSC102	*rad26*Δ::*HIS3*[a]	34
MGSC104	*rad7*Δ::*LEU2*[a]	38
MGSC106	*rad7*Δ::*LEU2 rad26*Δ::*HIS3*[a]	This study[b]
MGSC107	*rad16*Δ::*LEU2 rad26*Δ::*HIS3*[a]	This study[b]
MGSC108	*rad7*Δ::*LEU2 rad16*Δ::*URA3 rad26*Δ::*HIS3*[a]	This study[b]
MGSC126	*rad16*Δ::*LEU2*[a]	This study[b]
MGSC139	*rad14*Δ::*LEU2*[a]	This study[b]
MGSC140	*rad14*Δ::*LEU2 rad26*Δ::*HIS3*[a]	This study[b]

[a] The remainder of the genotype is that of W303-1B.
[b] Constructed as described in Materials and Methods.

a *rad26* disruption mutant is not more UV sensitive than a *RAD*$^+$ strain, in contrast to human CS-B cell lines.

It is possible that the defect in transcription-coupled repair in a *rad26* mutant is partly compensated for by global genome repair, explaining the absence of UV sensitivity in such a mutant. To examine this possibility, survival after UV irradiation of *rad7 rad26* and *rad16 rad26* double mutants was determined and compared with survival of various single mutants. Analysis of these mutants also allowed testing of the idea that NER is accomplished by the additive contribution of transcription-coupled repair and global genome repair. Removal of dimers from both individual strands of the active *RPB2* gene and the induced or repressed *GAL7* gene in the various mutants was determined to answer this question. The results provide more insight into the relationship between transcription-coupled and global genome repair, have implications for the role of the *RAD26* gene in transcription-coupled repair in *S. cerevisiae*, and may have implications for the molecular defect in CS.

MATERIALS AND METHODS

General procedures. All general procedures, including DNA purification, restriction enzyme digestion, cloning, PCR, and gel electrophoresis, were performed according to standard procedures (22). Plasmids were propagated in *E. coli* JM101 under appropriate antibiotic selection.

Yeast strains and media. The yeast strains used for this study are listed in Table 1. All strains were kept on selective YNB (0.67% yeast nitrogen base, 2% glucose, 2% Bacto Agar) supplemented with the appropriate markers. Cells were grown in complete medium (YEPD) (1% yeast extract, 2% Bacto Peptone, 2% glucose) at 28°C under vigorous shaking conditions. For induction of *GAL7*, glucose was replaced by galactose (28). Induction of *GAL7* on galactose-containing medium was confirmed by Northern (RNA) blot analysis.

Construction of disruption mutants. Yeast cells were transformed by electroporation (2,250 V/cm, 250 μF, 200 Ω). Cells were plated on YNB with the necessary amino acids and incubated at 28°C for 2 to 5 days. Successful disruption (21) was confirmed by Southern analysis.

Strain MGSC102 (*rad26*Δ::*HIS3* [34]) was transformed with *Bgl*I-linearized plasmid pRAD7Δ::LEU2 (38) to obtain strain MGSC106 (*rad7 rad26*).

Strains W303-1B and MGSC102 (*rad26*Δ::*HIS3*) were transformed with *Pvu*I-linearized plasmid pUB33 (gift of D. D. Bang), which contains the *LEU2* gene inserted in place of the *Hin*dIII fragment of *RAD16* (2), to obtain strains MGSC126 (*rad16*) and MGSC107 (*rad16 rad26*), respectively. These strains carry the same *rad16* deletion as strain W303236 (38), but with *LEU2* as a selectable marker instead of *URA3*.

MGSC105 (*rad7*Δ::*LEU2 rad16*Δ::*URA3* [38]) was transformed with linearized pPTZSHE6ScΔ::HIS3 (34) to generate strain MGSC108 (*rad7 rad16 rad26*).

W303-1B and MGSC101 (*rad26*Δ::*HIS3*) were transformed with *Sac*I-*Nco*I-digested pBM190 (gift of L. Prakash [3]) to generate strains MGSC139 (*rad14*) and MGSC140 (*rad14 rad26*), respectively.

UV survival curves. Yeast 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.

UV irradiation and DNA isolation. Yeast cells diluted in chilled phosphate-buffered saline were irradiated with 254-nm UV light (Philips T UV 30W) at a rate of 3.5 J/m^2/s. Cells were collected by centrifugation, resuspended in growth medium, and incubated for various times in the dark at 28°C prior to DNA isolation (26). DNA was purified on CsCl gradients (22).

Specific probes. Construction and isolation of single-stranded M13-derived probes recognizing the *RPB2* gene were performed as described before (38).

To construct strand-specific probes recognizing the *GAL7* gene, oligonucleotides 5′GGTTTTGCAATCGAGCCTGGTAG3′ and 5′GGCCAGATGGCCCAGTATG3′ were synthesized and PCR was performed on yeast strain W303-1B chromosomal DNA with these primers (35 cycles, annealing temperature of 55°C), generating a 1.6-kb fragment. This fragment was digested with *Acc*I, the site was filled with Klenow enzyme to generate a blunt end, and the fragment was digested with *Bgl*II. The resulting 1.1-kb blunt-*Bgl*II fragment was cloned in both orientations in M13 digested with *Hin*cII and *Bam*HI (M13mp18 and M13mp19).

Single-stranded DNA was isolated as described by Sambrook et al. (22) and used for primer extension to generate ^{32}P-labeled strand-specific probes as described earlier (34, 38).

Gene-specific repair assay. Genomic DNA was cut with restriction endonucleases *Pvu*I and *Pvu*II, generating a 5.2-kb *RPB2* fragment (30) and a 4.7-kb *GAL7* fragment. DNA samples were divided in two equal parts. One was incubated with T4 endonuclease V (isolated as described in reference 18), the other was mock treated, and both were loaded on denaturing agarose gels as described by Bohr et al. (4). After electrophoresis, the DNA was transferred to Hybond N+ (Amersham) and hybridized to strand-specific probes. After hybridization and data analysis, the probe was removed by alkaline washing, and subsequently the blot was hybridized to another probe. In this way, it was possible to determine dimer removal from both strands of the *RPB2* fragment and the *GAL7* fragment on every blot (four probes).

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 according to the Poisson distribution as described previously (4). After being scanned in the blot analyzer, autoradiographs were prepared from the Southern blots.

RESULTS

Survival of double mutants disturbed in transcription-coupled as well as global genome repair. To investigate whether the lack of UV sensitivity of a *rad26* mutant is due to compensation of the NER defect by global genome repair, we studied mutants that lack both *RAD26* and factors essential for global genome repair. Isogenic *rad26*, *rad7*, *rad16*, *rad7 rad26*, *rad16 rad26*, and *rad7 rad16 rad26* disruption mutants were constructed from the repair-proficient (*RAD*$^+$) strain W303-1B (see Materials and Methods and Table 1). Isogenic strains totally deficient in NER were constructed by disruption of the *RAD14* gene (3). The survival of the strains after irradiation with UV was measured.

From the results in Fig. 1, it is clear that although a single *rad26* mutant is not more UV sensitive than a *RAD*$^+$ strain (34), the *rad7 rad26* and *rad16 rad26* double mutants are more UV sensitive than single *rad7* or *rad16* mutants. In contrast, the survival of a *rad14 rad26* double mutant is identical to that of a completely NER-deficient *rad14* single mutant, indicating that no repair systems other than NER are impaired by a *rad26* mutation. Since *rad7*, *rad16*, and *rad14* mutants are in the same (*rad3*) epistasis group, the greater UV sensitivity of *rad7 rad26* and *rad16 rad26* mutants than of single *rad7* or *rad16* mutants is due to the combination of defects in global genome and transcription-coupled repair.

Strikingly, although an additive effect from disturbing both transcription-coupled and global genome repair is observed, the *rad7 rad26* and *rad16 rad26* double mutants are clearly not as UV sensitive as a completely NER-deficient *rad14* strain. This result indicates that there must be residual repair activity left in the double mutants.

Analysis of dimer removal from both strands of the *RPB2* gene. To examine the nature of the remaining repair activity in the double mutants, we analyzed dimer removal from the individual strands of an active gene with the method described by Bohr et al. (4). The results of the repair experiments using the *RPB2* gene are shown in Fig. 2.

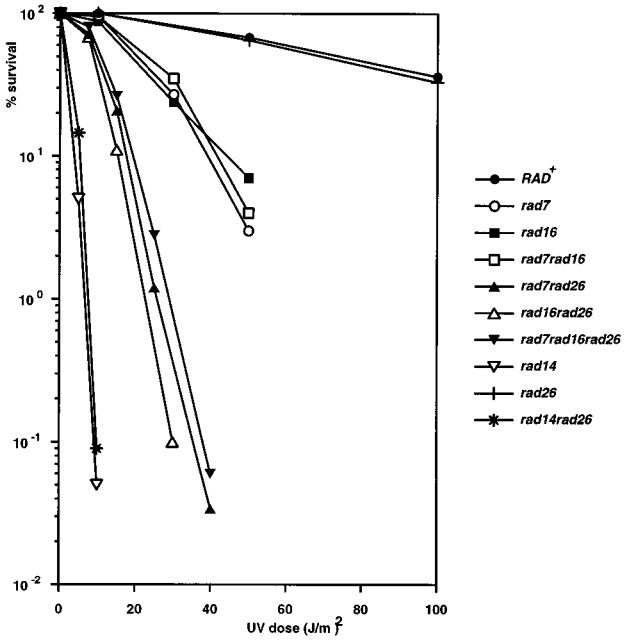

FIG. 1. UV survival of double mutants disturbed in global genome and transcription-coupled repair. The survival of strains W303-1B (*RAD*$^+$), MGSC102 (*rad26*), MGSC104 (*rad7*), W303236 (*rad16*), MGSC105 (*rad7 rad16*), MGSC106 (*rad7 rad26*), MGSC107 (*rad16 rad26*), MGSC108 (*rad7 rad16 rad26*), MGSC139 (*rad14*), and MGSC140 (*rad14 rad26*) after UV irradiation is depicted.

As we described before (34, 38), *rad7* or *rad16* single mutants are defective in repair of the nontranscribed strand of *RPB2*, while *RAD26* disruption leads to a strong decrease of the preferential repair of the *RPB2* transcribed strand (Fig. 2B).

Survival experiments suggested that global repair can contribute to removal of dimers from the transcribed strand when Rad26p is absent (see above). This was tested more directly by analyzing repair of *RPB2* in the *rad7 rad26* and *rad16 rad26* double mutants. Repair of the transcribed strand in these double mutants is less efficient than in the single *rad26* mutant (Fig. 2C), suggesting that *RAD7* and *RAD16* contribute to repair of the transcribed strand when transcription-coupled repair is hampered. Since the *rad7 rad26* and *rad16 rad26* mutants are not as UV sensitive as *rad14* strains, they should be able to repair at least part of their DNA. Figures 2A and C show that the transcribed strand and not the nontranscribed strand of *RPB2* is still repaired to a considerable extent in the *rad7 rad26* and *rad16 rad26* double mutants, suggesting that the residual repair activity in these mutants is transcription coupled.

Repair of the *GAL7* gene under induced and repressed conditions. To determine whether the residual repair activity in the *rad7 rad26* and *rad16 rad26* double mutants is indeed dependent on transcription, we analyzed strand-specific repair of the *GAL7* gene. This gene is repressed in medium containing glucose, whereas it is strongly induced in medium containing galactose (28). We studied repair of the *GAL7* gene in a *Pvu*I-*Pvu*II fragment that is comparable in size to the *RPB2* *Pvu*I-*Pvu*II fragment, enabling direct comparison of the rates of dimer removal from the *GAL7* and *RPB2* genes on the same blot, thus providing an internal control to exclude possible medium effects.

Figure 3A illustrates that in the *RAD*$^+$ strain, the template strand (the strand that is transcribed under induced conditions) is repaired faster than the nontranscribed strand when the gene is induced and that this difference is almost absent when the gene is repressed, which is consistent with earlier observations by Leadon and Lawrence (14).

Repair of the nontranscribed strand of *GAL7* is fully dependent on *RAD7* and *RAD16* (Fig. 3B), as expected and in agreement with the results obtained with *RPB2*. Also, the repair of the template strand confirms the results obtained with *RPB2*: under induced conditions with transcription-coupled repair active, *RAD7* or *RAD16* does not contribute to repair of this strand (Fig. 3B). Under repressed conditions, however, global genome repair does contribute to repair of the template strand, since in the *rad7* and *rad16* mutants, the repair of this strand is strongly inhibited (Fig. 3B). Under these conditions, there is still some residual repair of the template strand of the *GAL7* gene in the *rad7* and *rad16* mutants. This may be attributable to transcription-coupled repair as a result of some residual transcription, especially since it appears that this repair is dependent on *RAD26* (see below).

When the effect of *RAD26* disruption on the repair of the transcribed strand of *GAL7* was measured, a rather surprising result was obtained (Fig. 3C). In contrast to *RPB2*, for which a strong reduction in the repair rate of the transcribed strand is found (analyzed by using the same DNA on the same blot), the repair of the template strand of *GAL7* under induced conditions is nearly the same as in *RAD*$^+$ cells. Apparently the contribution of Rad26p to transcription-coupled repair can vary for different loci and is much more apparent for the *RPB2* gene than for *GAL7* under induced conditions. Under repressed conditions, both strands of the *GAL7* gene are repaired at the same rate in the *rad26* mutant (Fig. 3C), a result that was expected since under the same conditions almost no difference is observed between repair of both strands of *GAL7* in *RAD*$^+$ cells (Fig. 3A). This finding is consistent with the idea that in the absence of transcription, removal of dimers from both strands is performed by global genome repair that is independent of Rad26p.

When the global genome repair pathway is also impaired (in the *rad7 rad26* and *rad16 rad26* double mutants), the nontranscribed strand of *GAL7* is not repaired (Fig. 3D), as expected. Notably, the template strand of *GAL7* is still repaired under induced conditions, but no dimers are removed from this strand under repressed conditions (Fig. 3D). This finding demonstrates that repair of the *GAL7* template strand in the double mutants is dependent on transcription and moreover suggests that transcription per se can accomplish transcription-coupled repair independent of Rad26p. The residual repair of the template strand that was observed under repressed conditions in the *rad7* and *rad16* mutants (Fig. 3B) requires a functional *RAD26* gene, implying that Rad26p contributes to transcription-coupled repair of *GAL7* under repressed conditions.

Taken together, these results point to the existence of a Rad26p-independent mode of transcription-coupled repair and to locus heterogeneity with regard to the influence of Rad26p on transcription-coupled repair.

DISCUSSION

***RAD26* and transcription-coupled repair.** *rad26* disruption mutants are as UV resistant as *RAD*$^+$ strains. Possibly global genome repair can compensate for the loss of *RAD26* by ensuring repair of transcribed DNA (34). Here we show that this is indeed the case because there is a more than additive effect of mutations in *RAD26* combined with mutations in the *RAD7* and *RAD16* genes involved in global genome repair (38), implying that global genome repair factors are responsible for the

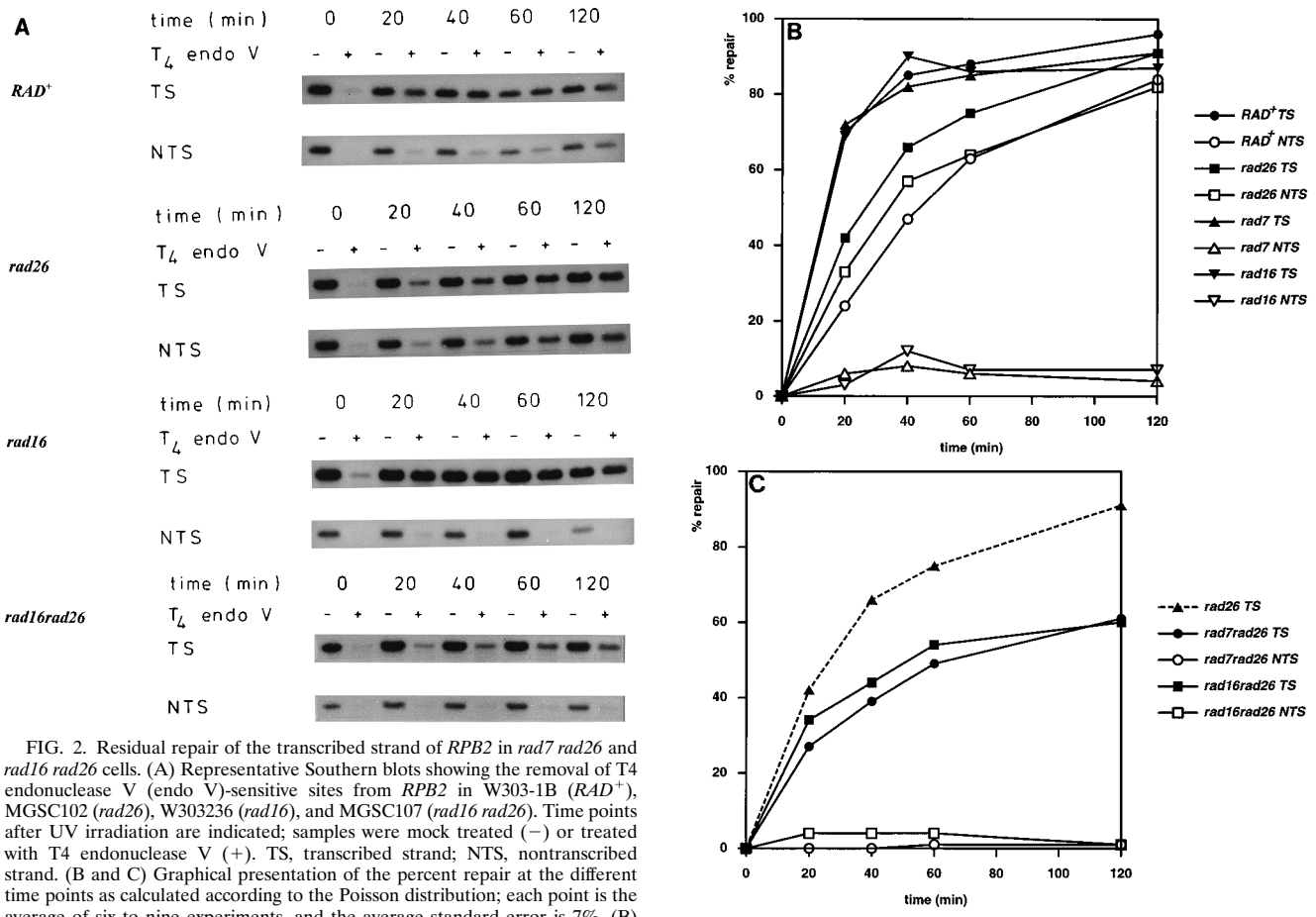

FIG. 2. Residual repair of the transcribed strand of *RPB2* in *rad7 rad26* and *rad16 rad26* cells. (A) Representative Southern blots showing the removal of T4 endonuclease V (endo V)-sensitive sites from *RPB2* in W303-1B (*RAD*$^+$), MGSC102 (*rad26*), W303236 (*rad16*), and MGSC107 (*rad16 rad26*). Time points after UV irradiation are indicated; samples were mock treated (−) or treated with T4 endonuclease V (+). TS, transcribed strand; NTS, nontranscribed strand. (B and C) Graphical presentation of the percent repair at the different time points as calculated according to the Poisson distribution; each point is the average of six to nine experiments, and the average standard error is 7%. (B) Repair of both strands of *RPB2* in *RAD*$^+$, *rad26*, *rad7*, and *rad16* cells confirms our earlier results (34, 38). (C) Repair of *RPB2* in *rad7 rad26* and *rad16 rad26* double mutants. For comparison, data for repair of the transcribed strand in a *rad26* mutant are also depicted (dashed line). The degree of repair of both strands in the *rad7 rad16 rad26* triple mutant is identical to the degree of repair in the *rad7 rad26* and *rad16 rad26* double mutants.

lack of UV sensitivity of a *rad26* mutant. Human CS-B cells, in contrast, are markedly UV sensitive. A possible explanation for this difference is that the global genome repair process may be more efficient in *S. cerevisiae* than in higher eukaryotic species with a more complex genome. The contribution of Rad26p to survival after UV exposure that is revealed in the double mutants stresses the involvement of the *RAD26* gene in general transcription-coupled repair and unmasks the contribution of this process to cellular UV resistance. Therefore, these data strengthen the correspondence between *RAD26* and *CSB*.

Although the foregoing and previous findings unequivocally establish the involvement of Rad26p in transcription-coupled repair, double mutants lacking *RAD26* and global genome repair are significantly less UV sensitive than completely NER-deficient mutants. Furthermore, analysis of gene- and strand-specific repair in the *RPB2* and *GAL7* genes reveals that these mutants are, to a variable extent, still capable of repairing the transcribed strand only. The experiments with the inducible *GAL7* gene strongly suggest that this repair is transcription dependent. It has already been demonstrated that strand-specific repair of *RPB2* and *GAL7* requires functional RNA polymerase II (14, 30), pointing to a direct role for the transcription machinery in transcription-coupled repair. Since strains that lack Rad26p and global genome repair display residual repair of the transcribed strand selectively, this repair must be dependent on transcription. We conclude that part of the transcription-coupled repair is Rad26p independent. Incomplete inactivation of transcription-coupled repair also explains the slight but reproducible preferential repair of the transcribed strand over the nontranscribed strand of the *RPB2* gene in the *rad26* mutant (34) (Fig. 2B).

The effect of *RAD26* disruption on repair of the template strand of *GAL7* under conditions such that the transcription rate is high (28) is very small or absent (Fig. 3C), in contrast to the clear and significant effect on transcription-coupled repair of *RPB2* (Fig. 2B) and the *PHO5 PHO3* locus (unpublished results). Apparently, Rad26p-independent transcription-coupled repair is more efficient for induced *GAL7* than for the *RPB2* gene. Since deletion of *RAD26* has some effect on *GAL7* repair under repressed conditions (Fig. 3B and D), it might be possible that the high efficiency of *RAD26*-independent transcription-coupled repair in *GAL7* is related to a high transcription rate of the *GAL7* gene under induced conditions. Although without further experimentation this hypothesis remains merely speculative, it is interesting that a similar observation has been reported for *E. coli*: *mfd* mutants (lacking TRCF [24]) are still able to preferentially repair the transcribed strand of the *lacZ* gene in vivo when this gene is induced with isopropylthiogalactopyranoside (IPTG) but not when the gene is transcribed at a low rate (13).

The observation of Rad26p-independent transcription-cou-

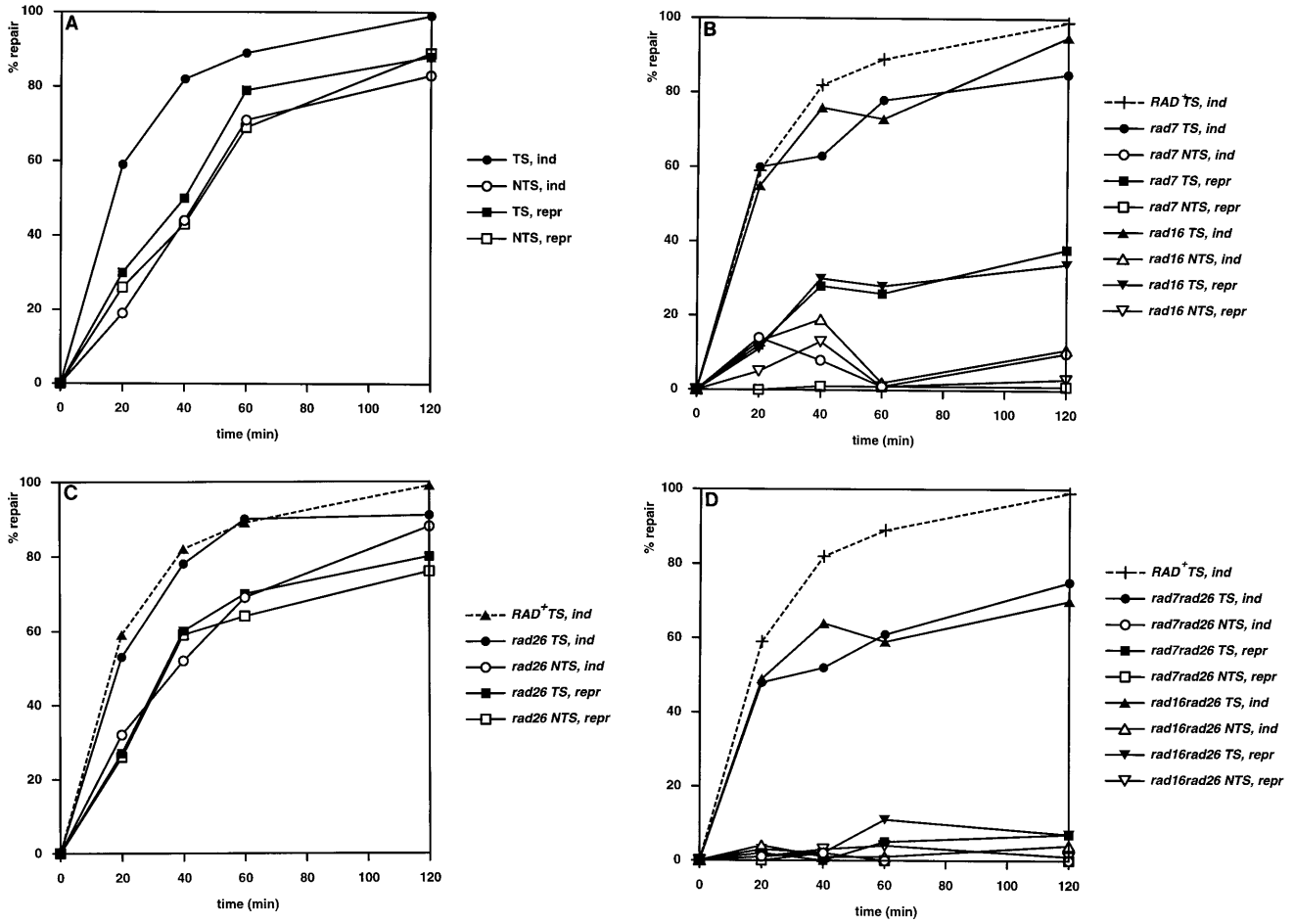

FIG. 3. *GAL7* is repaired only under induced conditions in *rad7 rad26* and *rad16 rad26* mutants. Repair of the *GAL7* gene was calculated according to the Poisson distribution for each time point; the data are from three to four experiments, and the average standard error is 6%. TS, transcribed (template) strand; NTS, nontranscribed (nontemplate) strand; ind, induced conditions (galactose); repr, repressed conditions (glucose). (A) Repair of *GAL7* in RAD^+ cells. (B) Repair of *GAL7* in *rad7* and *rad16* cells. For comparison, data for the transcribed strand under induced conditions in RAD^+ cells are also depicted (dashed line). (C) Repair of *GAL7* in *rad26* cells. For comparison, data for the transcribed strand under induced conditions in RAD^+ cells are also depicted (dashed line). (D) Repair of *GAL7* in *rad7 rad26* and *rad16 rad26* cells. For comparison, data for the transcribed strand of RAD^+ cells under induced conditions are also depicted (dashed line). No difference in repair of the constitutively expressed *RPB2* gene was observed for the same strain on medium containing glucose compared with medium with galactose. Therefore, all differences observed in repair of *GAL7* in cells grown in both media are specifically due to the induced and repressed states of the *GAL7* gene in these cells and not due to general repair differences as a consequence of the different media.

pled repair suggests that Rad26p has an auxiliary function important for the efficiency of transcription-coupled repair but is not essential for this process. It is therefore not likely that Rad26p is the yeast counterpart of the *E. coli* TRCF, a protein that couples repair to transcription by specifically targeting repair enzymes to lesions that obstruct RNA polymerase (25). If Rad26p is a transcription-repair coupling factor, then either such a coupling factor is not essential in yeast cells or a protein other than Rad26p can independently perform transcription-repair coupling. A possible candidate for such a redundant factor is the yeast homolog of CSA, although in human cell lines defects in either *CSA* or *CSB* lead to abolishment of transcription-coupled repair (11, 35). Transcription-repair coupling may have different molecular backgrounds in prokaryotes and eukaryotes (9). The necessity for a TRCF as found in *E. coli* (25) might in eukaryotes be obviated by the intimate association of several NER enzymes with basal transcription factors (6, 23, 29). If backtracking of a blocked RNA polymerase is a prerequisite for eukaryotic transcription-coupled repair (5), Rad26p is not essential for this process. The recent notion that CS may be caused by defects in the transcription process, indirectly leading to defects in transcription-coupled repair (11), should also be considered. Transcriptional defects in *rad26* cells could account for the defect in transcription-coupled repair that we observe. However, we did not detect defects in growth rates (34) or transcription of *RPB2* and *GAL7* in our *rad26* mutants (unpublished data), although subtle transcriptional defects cannot be excluded.

Global genome repair. *RAD7* and *RAD16* are essential for global genome repair in yeast cells. Silenced DNA and the nontranscribed strands of the *RPB2* and *GAL7* genes are not repaired in *rad7* and *rad16* mutants, but the transcribed strand is repaired with the same efficiency as in repair-proficient cells. However, inactivating global genome repair leads to a repair defect for the template strand of *GAL7* when transcription is nearly absent (Fig. 3B), proving that global genome repair is capable of functioning on a strand that is a template for transcription-coupled repair under induced conditions. The diminished repair rate of the transcribed strand in *rad7 rad26* and *rad16 rad26* double mutants compared with *rad26* single mu-

tants probably also reflects a contribution of *RAD7* and *RAD16* to repair of the transcribed strand. Therefore, the term global genome repair is really warranted for this system, which was previously implicated only in repair of nontranscribed DNA (38).

How the proteins involved in global genome repair act at the molecular level is still unknown. One could envisage that the chromatin context of the DNA damage in eukaryotes necessitates such factors to make the damaged DNA a substrate for the actual incision enzymes. Interestingly, Rad16p shares functional domains with Swi2p/Snf2p (2), a factor that may be involved in suppression of chromatin-mediated repression of transcription (20), while Rad7p has been shown to interact with Sir3p (19), a putative component of silent yeast chromatin (10). Rad7p and Rad16p seem dispensable for a reconstituted NER reaction on naked plasmid DNA (8), corroborating a role for these proteins in vivo on DNA packed into chromatin. However, our *rad7* and *rad16* mutants are completely defective for NER in a cell-free system that is also devoid of transcriptional activity (39).

The reconstitution of in vitro NER reactions (1, 8, 17) is highly informative with regard to the biochemistry of NER. Nevertheless, our results underscore the importance of analyzing dimer removal in vivo to be able to appreciate the complexity of NER and identify components that play a role in the organization of NER. Since the incision and subsequent steps of NER are probably not different for the two strands (1, 8, 17), we hypothesize that dimers can be removed only when they are made accessible for repair enzymes either by transcription or by the global genome repair proteins. Absence of both transcription and one of the global repair proteins, Rad7p or Rad16p, leads to complete inactivation of NER in vivo, as a mutation in one of the core components of NER does.

In summary, we show in this report that transcription-coupled repair and global genome repair are partially overlapping subpathways of NER, we demonstrate a role for the *RAD26* gene in UV survival, and we infer the existence of Rad26p-independent transcription-coupled repair.

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