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Defective nucleotide excision repair in yeast *hpr1* and *tho2* mutants

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ABSTRACT

Nucleotide excision repair (NER) and transcription are intimately related. First, TFIIH has a dual role in transcription initiation and NER and, secondly, transcription leads to more efficient repair of damage present in transcribed sequences. It is thought that elongating RNAPII, stalled at a DNA lesion, is used for the loading of the NER machinery in a process termed transcription-coupled repair (TCR). Non-transcribed regions are repaired by the so-called global genome repair (GGR). We have previously defined a number of yeast genes, whose deletions confer transcription-dependent hyper-recombination phenotypes. As these mutations cause impairment of transcription elongation we have assayed whether they also affect DNA repair. We show that null mutations of the *HPR1* and *THO2* genes, encoding two prominent proteins of the THO complex, increase UV sensitivity of yeast cells lacking GGR. Consistent with this result, molecular analyses of DNA repair of the *RPB2* transcribed strand using T4 endo V show that *hpr1* and *tho2* do indeed impair TCR. However, this effect is not confined to TCR alone because the mutants are slightly affected in GGR. These results indicate that THO affects both transcription and NER. We discuss different alternatives to explain the effect of the THO complex on DNA repair.

INTRODUCTION

Nucleotide excision repair (NER) is an evolutionarily conserved DNA repair pathway that maintains the genome free of a wide variety of lesions, such as those induced by UV light and some chemical agents (1,2). In yeast, more than 25 proteins participate in this multi-step process (2).

One of the most intriguing aspects of all forms of DNA repair is the mechanism by which a DNA lesion is detected. In NER, even though the proteins that recognize the lesion (UvrA in *Escherichia coli* and Rad4/Rad23 and XPC/HHR23B in yeast and humans) have been identified, it is not clear how such proteins are targeted to the site of the lesion. According with the mechanism of lesion detection, NER can be divided into two subpathways: global genome repair (GGR) and transcription-coupled repair (TCR). The first one acts in all regions of the genome irrespective of whether or not they are transcribed. In yeast, GGR requires Rad7, a protein carrying leucine-rich repeats and Rad16, a member of the SWI2/SNF2 subfamily of putative helicases. These proteins presumably act in a complex (3,4) that might be required in chromatin remodeling to facilitate damage detection by Rad4/Rad23 (5–7). TCR depends on the product of the Rad26 gene and is confined to the transcribed strands (TS) of active genes (8,9).

TCR takes advantage of the incapacity of the RNA polymerases (RNAP) to pass particular DNA lesions such as UV-induced cyclobutane-pyrimidine dimers (CPDs). RNAPII, stalled at a DNA lesion, is used for the loading of the NER machinery at the site of the lesion. It has been shown that a stalled bacterial RNAP at sites of base damage in the TS inhibits the repair of CPDs by the UvrABC NER system *in vitro* (10). This suggests that TCR requires the displacement of RNAP from the damaged site to allow NER, a reaction mediated by a transcription-repair coupling factor (TRCF) (11).

In eukaryotes, in which TCR was first discovered (12,13), TRCF candidates are the human Cockayne syndrome B and A proteins, or their respective yeast orthologs Rad26 and Rad28. While human cells deficient in CSB and CSA and yeast *rad26* mutants show TCR defects (9,14), *rad28* mutants are TCR proficient (15). CSB and Rad26 belong to the SWI2/SNF2 subfamily of putative helicases. CSB, as well as bacterial TRCF, have been shown to have ATPase activity (16,17), but no helicase activity has been detected for either protein. However, the function of CSB/Rad26 has not yet been established. The observations that CSB-deficient cells display reduced transcription levels (18), that purified CSB enhances RNAPII-mediated elongation *in vitro* (19,20) and that CSB resides in a RNA polymerase II (RNAPII)-containing complex (21) have raised the possibility that CSB/Rad26 is a transcription elongation factor. The TCR phenotype of CSB/Rad26-deficient cells could be a consequence of impaired transcription elongation. The observations that α-amanitin, an inhibitor of transcription elongation, inhibits TCR in CHO cells (22) and that the absence of the Spt4 elongation factor bypasses the requirement

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Table 1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Source/reference
W303-1A	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein (New York)
W839-5D	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad1Δ::LEU2*	R. Rothstein (New York)
U768-1C	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3*	R. Rothstein (New York)
W303-236	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad16Δ::URA3*	(37)
MGSC97	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad7Δ::URA3*	(37)
MGSC102	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad26Δ::HIS3*	(9)
SChY58a	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::*kanMX4	S. Chávez (Seville)
RK2-6C	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tho2Δ::*kanMX4	(26)
TR16-11A	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tho2Δ::*kanMX4 *rad16Δ::URA3*	This work
TR16-9B	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tho2Δ::*kanMX4 *rad16Δ::URA3*	This work
TR7-8A	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tho2Δ::*kanMX4 *rad7Δ::URA3*	This work
TR7-1B	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tho2Δ::*kanMX4 *rad7Δ::URA3*	This work
TR26-1C	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 tho2Δ::*kanMX4 *rad26Δ::HIS3*	This work
HR7-7B	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad7Δ::URA3*	This work
HR7-5D	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad7Δ::URA3*	This work
HR16-3D	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad16Δ::URA3*	This work
HR16-2C	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad16Δ::URA3*	This work
R267-10A	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 rad26Δ::HIS3 rad7Δ::URA3*	This work
MU26-10B	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad26Δ::HIS3*	This work
HR2616-2A	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad26Δ::HIS3 rad16Δ::URA3*	This work
HR2616-7C	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad26Δ::HIS3 rad16Δ::URA3*	This work
HR267-2B	*MAT**a** ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad26Δ::HIS3 rad7Δ::URA3*	This work
HR267-6A	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 hpr1Δ::HIS3 rad26Δ::HIS3 rad7Δ::URA3*	This work

for Rad26 in yeast TCR would be consistent with the idea that CSB/Rad26 is not the eukaryotic TRCF (23).

We have identified four genes, *HPR1*, *THO2*, *MFT1* and *THP2*, whose null mutations confer a strong hyper-recombination phenotype between repeats that is dependent on transcription elongation (24–26). These genes encode proteins that belong to the same protein complex, termed THO (24), which connects transcription with mitotic recombination. Mutations in these genes impair transcription elongation (27,28). Such an impairment is particularly strong in long and G+C-rich DNA sequences (29). As a consequence of the transcription elongation defect, mutants of the THO complex show an increased recombination frequency between direct repeats (24–31), and a high frequency of chromosome and plasmid loss (26,27,30). We have hypothesized that THO may have a role in transcription elongation. In the mutants of the THO complex, transcription elongation could be impaired leading to the formation of recombinogenic structures (27,28).

Considering the existing connection between transcription and repair and the connection between transcription and genetic instability provided by the THO complex, we have asked whether mutation of *HPR1* and *THO2*, the two most prominent components of the THO complex, impair NER. We show that this is the case. *hpr1* and *tho2* are affected in TCR, but additionally also slightly in GGR. Altogether, our results support a general effect of the THO complex in NER.

MATERIALS AND METHODS

Yeast strains

The yeast strains used in this study, all isogenic, are listed in Table 1.

UV survival curves

Yeast cells were grown in YEPD-rich medium (32) to an OD_{600} of 1. Different dilutions were plated on YEPD and irradiated with the different UV doses with a 254 nm UV lamp (Philips T UV 30 W). Plates were incubated for 3 days at 30°C in the dark before colonies were counted and survival was calculated. All survival curves shown represent the average of three independent experiments.

UV irradiation and DNA isolation

Yeast cultures were grown in 500 ml of YEPD overnight to a final OD_{600} of 1.8. Cells were collected by centrifugation, resuspended in 1.6 l chilled phosphate-buffered saline (PBS) and irradiated with a 254 nm UV lamp at 25 J/m^2. Subsequently, cells were incubated in YEPD for various time courses in the dark at 30°C. After this, cell pellets collected by centrifugation were frozen in liquid nitrogen and maintained at –80°C until DNA was isolated.

For DNA isolation we followed a modified version of previously published procedures (33). Pellets were resuspended to a final concentration of 1.5–2 × 10^9/ml in NIB

pH 7.2 (17% glycerol, 50 mM MOPS, 150 mM KAc, 2 mM MgCl, 0.5 mM spermidine and 0.15 mM spermine) with 3 mg/ml Zymoliase-20T and incubated at room temperature for 20 min. Cellular suspensions were diluted seven times with chilled water and centrifuged to collect nuclei. Pellets were resuspended in 5.4 ml of TE (50 mM Tris–HCl pH 8, 20 mM EDTA) and mixed with 600 µl of 10% SDS. After phenol extraction and ethanol precipitation, DNA was finally purified on CsCl gradients and dialyzed in TE (34).

Gene- and strand-specific DNA repair assays

Five micrograms of genomic DNA were digested with *Pvu*I and *Pvu*II, generating a 5.2 kb *RPB2* fragment (35) and a 4.7 kb *GAL7* fragment (36), with *Hae*II, which generates a 3.5 kb *MAT*α fragment and a 4.1 kb *HML*α fragment, or with *Hin*dIII, which generates a 4.3 kb *MATa* and a 5.1 kb *HMRa* fragment (37). In each case, DNA samples were divided in two halves. One was incubated with T4 endonuclease V (T4 endo V) at 37°C for 2 h. After electrophoresis and Southern blot transfer to Hybond N^+, samples were hybridized at 65°C (7% SDS and 0.5 M phosphate buffer pH 7) with ^{32}P-labeled DNA probes. The same filters were rehybridized up to four times, after removing probes by harsh treatment with boiling 0.1% SDS. Bands were quantified in a Fujix FLA3000. All values of the bands corresponding to the T4-untreated samples were divided by the T4-mock sample of zero time. These values were multiplied by their corresponding values of the T4-treated sample and subtracted from the T4-treated zero time to eliminate the basal background. The final percentage of repair was calculated by dividing these resulting values by the value of the T4-mock-treated zero time.

RPB2 and *GAL7* strand-specific probes were obtained by primer extension from M13 derivative vectors as described (37). 634 bp Yα-specific probes were obtained by PCR with oligonucleotides GCCAAACTGTGAGTAATATGC and TCATCTGTGATTTGTGGATTT and labeled with ^{32}P-dCTP. 602 bp Ya-specific probes were generated by PCR with primers ACCCGACTATGCTATTTTAAT and GGGGAAACTGTATAAAACTTC. All results shown correspond to the average of two to four independent experiments.

Northern analyses

For determination of RNA levels after UV treatment, yeast cells were grown at 30°C to an OD_{600} of ~0.5 in 100 ml YEP–3% glycerol–2% lactate, harvested by centrifugation and resuspended in 100 ml of PBS pH 7. Half of the culture was irradiated with a 254 nm UV light lamp at 70 J/m^2 and the other half was used as the unirradiated control. Each half was then grown at 30°C in 60 ml YEP–2% galactose medium. At the indicated time 10 ml aliquots were taken, collected by centrifugation, frozen in liquid nitrogen and maintained at –80°C until RNA extraction. Ten micrograms of each RNA sample were subjected to northern analysis following previously published procedures (27). Filters were first hybridized with a 0.75 kb *Pvu*II/*Ava*I *GAL1* probe and then re-hybridized with a 589 bp DNA internal fragment obtained by PCR as described (27). mRNA levels were quantified in a Fujix FLA3000 analyzer and are given as arbitrary units. All values were normalized with respect to the 28S rRNA. Northern analysis of *RPB2* was performed with RNA isolated from mid-log phase cultures according to standard procedures (34).

RESULTS

Increased UV sensitivity of *hpr1* and *tho2* mutant strains that also lack GGR

The *hpr1* and *tho2* mutations confer a defect in transcription elongation that causes a strong transcription-dependent hyper-recombination phenotype between DNA repeats. As NER consists of two subpathways, one of which, TCR, relies on an elongating RNAPII to detect DNA lesions, we decided to assay if *hpr1* and *tho2* mutations cause a defect in TCR. These mutations by themselves do not confer a DNA repair defect detectable as a UV-sensitivity phenotype (see Fig. 1) (25). As can be seen in Figure 1, the same UV-sensitivity curves were obtained in wild-type, *hpr1*, *tho2* and *rad26* strains, impaired in TCR, as well as in double mutant combination *hpr1 rad26* and *tho2 rad26*. As the TCR defect of *rad26* strains is only observed as a UV-sensitivity phenotype when GGR is also abolished, we decided to construct double mutant strains *hpr1 rad7*, *tho2 rad7*, *hpr1 rad16* and *tho2 rad16*. As shown in Figure 1 the viability upon UV irradiation of these double mutants was significantly reduced below the levels of the single mutants *rad7* and *rad16*. The survival curves were similar to those of *rad7 rad26* and *rad16 rad26* double mutants. This result indicates that both *hpr1* and *tho2* impair NER in the absence of GGR. In addition, triple mutants affected in *rad26*, *hpr1*/*tho2* and *rad7*/*rad16* display an increased UV sensitivity as compared with the double mutants *hpr1*/*tho2 rad7*/*rad16* or *rad26 rad7*/*rad16* (Fig. 2). Thus, Rad26 and Hpr1/Tho2 play different roles in TCR.

RNA synthesis is significantly reduced in wild-type, *rad26* and *tho2* cells after UV irradiation

In yeast, RNA synthesis of induced genes is initially inhibited after UV irradiation (38), probably a consequence of the inability of the RNAPII to pass through CPDs accumulated in the TS of genes. To determine whether the ability of resuming RNA synthesis after UV irradiation in cells affected in TCR is impaired, we determined the ability of *rad26* and *tho2* cells to transcribe the endogenous *GAL1* gene after irradiation with 70 J/m^2 UV. As can be seen in Figure 3, even in the absence of UV irradiation the *rad26* and *tho2* mutants display reduced transcription rates. Furthermore, after UV irradiation, there was a significant retardation in the kinetics of activation of *GAL1* in all strains. Whereas non-irradiated *rad26* cells reached 59% of the RNA levels of wild-type cells, this value was only 11% in non-irradiated *tho2* cells. Consistent with previous observations (26,39), our results reflect an intrinsic defect of both types of mutants in transcription, regardless of UV damage.

T4 endo V analysis of repair in the TS and non-transcribed strands (NTS) of the constitutively expressed gene *RPB2*

To determine, at the molecular level, whether *hpr1* and *tho2* impair TCR we analyzed the ability of *hpr1* and *tho2* cells to repair the TS and NTS of the constitutively expressed *RPB2* gene. The kinetics of repair of UV-induced lesions was followed by T4 endo V treatment. This methodology previously established that both wild-type and *rad7* cells show the same rates of repair in the TS of *RPB2*, whereas *rad26* cells

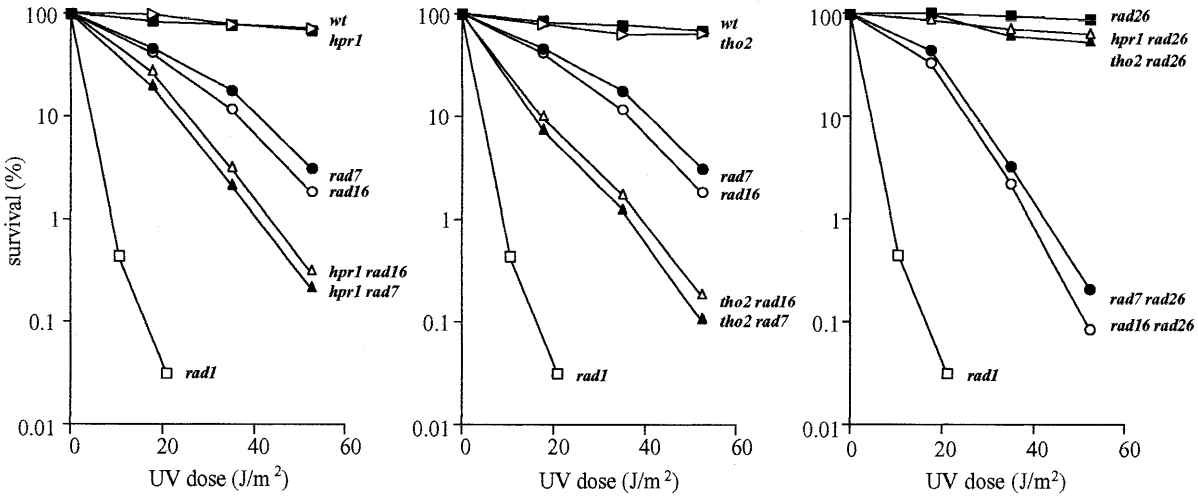

Figure 1. UV sensitivity curves of isogenic W303 yeast strains carrying different single and double combinations of the *hpr1*, *tho2*, *rad26*, *rad7* and *rad16* null mutations. The isogenic *rad1* strain W839-5D lacking NER was used as a negative control. Strains used are those listed in Table 1. The experiments were repeated two or three times for each genotype, giving similar results with standard deviations below 10% (data not shown). Only average values are shown.

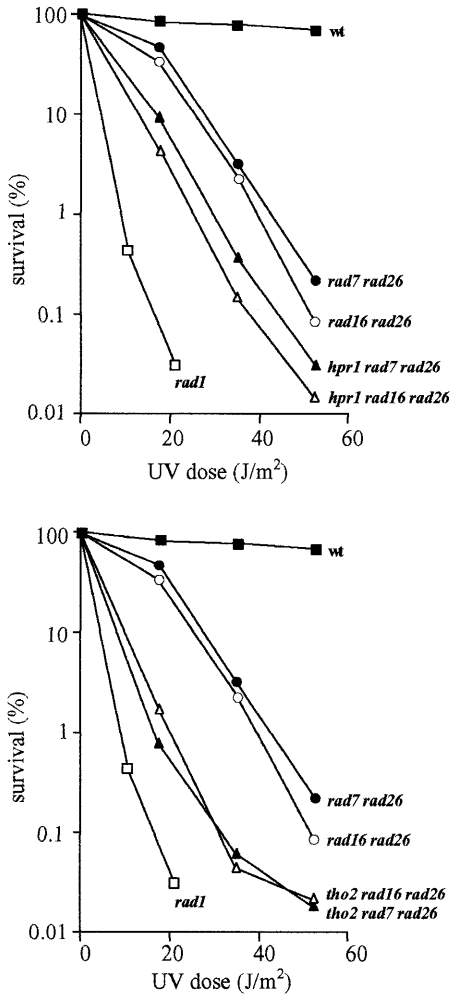

Figure 2. UV sensitivity curves of isogenic W303 yeast strains carrying different combinations of the *hpr1*, *tho2*, *rad26*, *rad7* and *rad16* null mutations compared with the appropriate controls. Other details as in Figure 1 legend.

show reduced rates (9,37). In addition, it is known that wild-type and *rad26* cells show the same kinetics of repair in the NTS of *RPB2*, whereas *rad7* cells show no repair (9,37). We have confirmed these results in our W303 isogenic strains (data not shown).

Figure 4 shows that in both the *hpr1* and *tho2* mutants there is a defect in the repair of the TS, especially observed as a reduction in the initial rate of repair. The defect in the repair of the TS of *RPB2* in both the *hpr1* and *tho2* mutants is as strong as in *rad26*, although in *rad26* cells this effect is persistent and never reaches wild-type levels of DNA repair.

Surprisingly, *hpr1* and *tho2* mutants showed also reduced kinetics of repair of the NTS of *RPB2*, although less pronounced than that displayed by *rad7* cells. Only at 120 min, both *hpr1* and *tho2* mutants reached wild-type levels of repair (Fig. 4). Therefore, *hpr1* and *tho2* mutants show delayed kinetics of repair of both the TS and the NTS of *RPB2*.

Transcription levels of *RPB2* are reduced in *rad26, rad7, hpr1* and *tho2* cells

Given that *hpr1* and *tho2* mutants are affected in DNA repair and transcription we decided to determine the levels of transcription of *RPB2* in all mutants analyzed (Fig. 5). Northern analysis shows that in all strains, including *rad7*, there was a 40–50% reduction in the overall levels of *RPB2* RNA. As expected, transcription of *RPB2* was impaired in *hpr1* and *tho2* cells, as well as in *rad26*. The unexpected reduced levels of transcription of *RPB2* observed in *rad7* cells suggest that the delayed kinetics of TCR observed in *hpr1* and *tho2* might not be caused by a defect in transcription. With similar reduction in transcription efficiency, *rad7* has no effect in the repair of the TS of *RPB2*.

Besides the transcriptional effect of *hpr1* and *tho2* on transcription elongation (26,27), it is worth noting that the expression levels of *RPB2*, *GAL1* and *GAL7* genes in *rad26* mutants were below 40% of the wild-type levels (Figs 3 and 5; data not shown). This result is consistent with a putative role in transcription elongation of Rad26/CSB (16,22,39). However, transcription of *RPB2* is also affected in *rad7* mutants (Fig. 5),

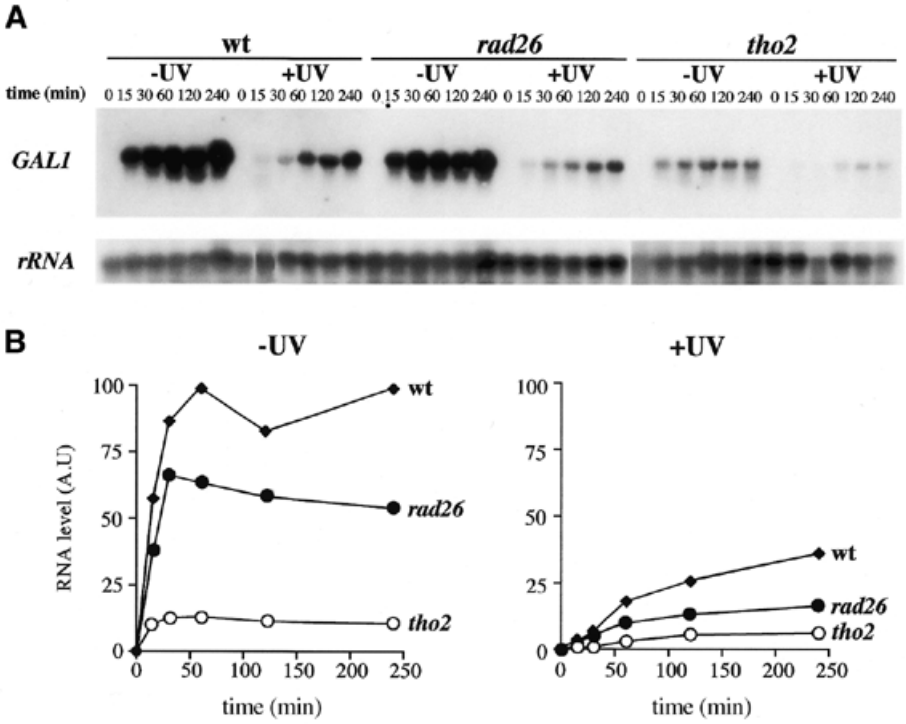

Figure 3. Kinetics of transcription activation of the endogenous *GAL1* gene upon UV irradiation in wild-type, *rad26* and *tho2* strains. (**A**) Northern analysis of *GAL1* at different times of transcription activation by addition of 2% galactose to YEP–3% glycerol–2% lactate mid-log phase cultures whether or not irradiated with 70 J/m^2. (**B**) Kinetics of transcription activation of *GAL1* as determined by quantification of the northern analysis. All data were normalized with respect to rRNA values.

but there is no evidence that the role of Rad7 is related to transcription. Therefore, DNA lesions might cause the low transcriptional levels observed in untreated *rad26* and *rad7* cells. If the assumption is correct, it could also imply that Rad26 may not necessarily be directly involved in transcription elongation, in contrast to what has been recently suggested (39).

T4 endo V analysis of UV-damage repair in *GAL7* and *HMLα* and *HMRa* loci under repressed conditions

As the analysis of *RPB2*, a constitutively expressed gene, indicates that *hpr1* and *tho2* cells are affected in the kinetics of repair of both the TS and the NTS, we analyzed whether repair of non-transcribed genes was also affected. We first determined the kinetics of repair of both strands of the *GAL7* gene under repressed conditions. As expected, the efficiency of TCR proficient *rad7* cells to repair the TS of the repressed *GAL7* gene was reduced as compared with its ability to repair the TS of the constitutively expressed *RPB2* gene (Fig. 6; compare with Fig. 4). Interestingly, there was still some repair of the TS of *GAL7* in *rad7* cells (Fig. 6) (40). This repair is dependent on *RAD26* and it has been interpreted as residual transcription at repressed condition (40), though we have not been able to detect any transcript by northern analysis (data not shown). The *hpr1* and *tho2* mutants show a kinetics of repair of the TS and NTS strands of *GAL7* similar to that observed in *RPB2* (compare Figs 4 and 6).

We also analyzed GGR in the silenced *HMLα* and *HMRa* loci that lack transcription due to silencing. As can be seen in Figure 7, repair is completely abolished in *rad7* cells, supporting a lack of transcription and associated TCR in these loci and the dependence of GGR on Rad7. As in the *RPB2* and *GAL7* genes, *hpr1* and *tho2* mutants show a delay in their capability to repair the silenced *HMLα* and *HMRa* loci. These results suggest that in addition to an effect in TCR, observed at both genetic and physical levels, *hpr1* and *tho2* are also affected in GGR. This effect, although detectable at the molecular level, apparently does not result in an UV-sensitivity phenotype of *hpr1*/*tho2* single mutants or *rad26 hpr1*/*tho2* double mutants.

DISCUSSION

We have shown that *hpr1* and *tho2* cells, impaired in transcription elongation and having a strong transcription-dependent hyper-recombination phenotype, are impaired in NER. The *hpr1* and *tho2* mutations confer UV sensitivity when combined with either *rad7* or *rad16*, which abolish GGR. Molecular analyses of DNA repair with T4 endo V indicate that *hpr1* and *tho2* cells are impaired in their ability to repair the TS of *RPB2* similarly to *rad26* cells. In contrast to *rad26* cells, *hpr1* and *tho2* also show repair defects of non-transcribed DNA, although at levels that are only detected at the molecular level.

Hpr1 and Tho2 are part of the THO complex, which connects transcription to genomic instability. Our observations that *hpr1* and *tho2* reduce UV survival only in strains impaired in GGR, and impair the repair of the constitutively expressed *RPB2* gene upon UV irradiation, as determined by T4 endo V analysis (Fig. 4) indicate that the THO complex modulates or has a role in TCR. However, in contrast to *rad26* mutations, *hpr1* and *tho2* have a slight effect on GGR. Thus, defective repair is also physically detected in the NTS of *RPB2*, in the repressed *GAL7* gene and in the silenced *HMLα* and *HMRa*

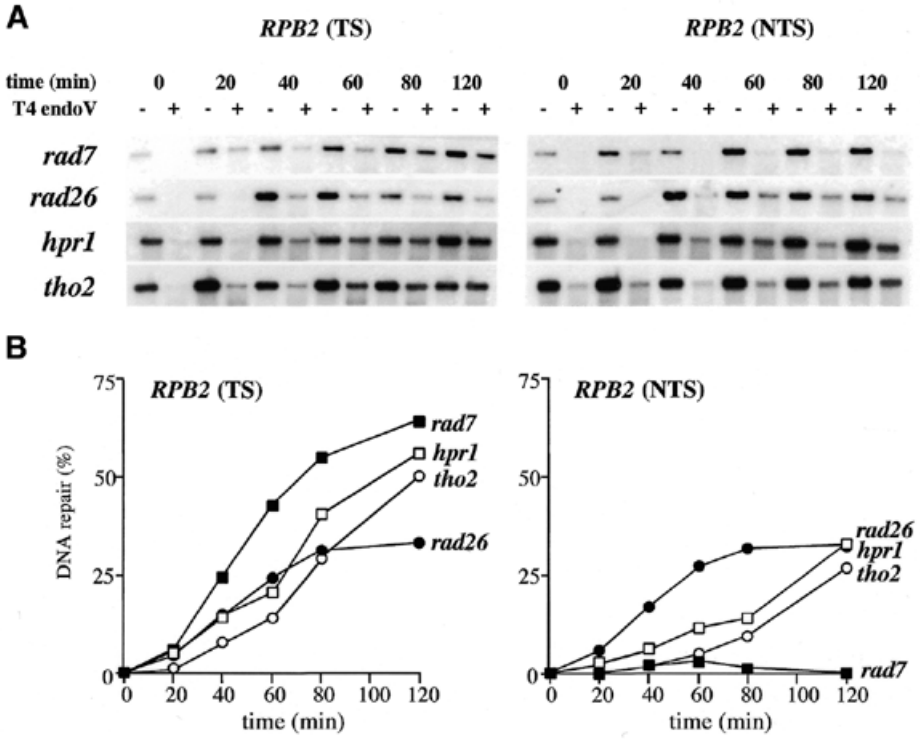

Figure 4. T4 endo V analysis of DNA repair at the endogenous *RPB2* gene. (**A**) Analysis of repair of the TS and NTS of *RPB2*. Cells obtained from mid-log phase YEPD cultures were irradiated with 25 J/m^2 UV and allowed to recover in YEPD-rich medium for different times to permit removal of lesions by NER. DNA was mocked (–) or T4 endo V treated (+), electrophoresed, blotted and hybridized with the appropriate *RPB2* single stranded probes obtained by primer extension. (**B**) Kinetics of repair as determined from the quantification analysis of the hybridization experiments. Strains *rad7* and *rad26* are shown as controls for wild-type TCR and wild-type GGR, respectively. The experiments were repeated four times for each genotype, giving similar results with standard deviations below 20%. Only average values are shown.

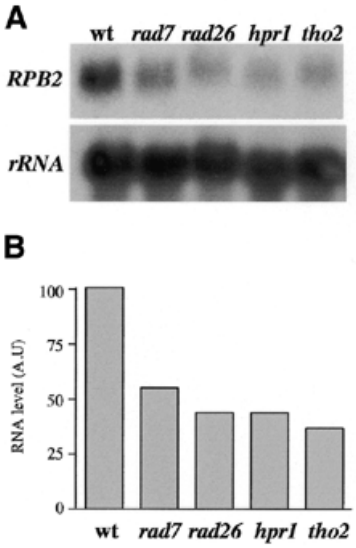

Figure 5. (**A**) Northern analysis of the endogenous *RPB2* gene in wild-type, *rad7*, *rad26*, *hpr1* and *tho2* strains. (**B**) Transcription levels of *RPB2* as determined from the quantification analysis of the northern analysis. Details as in Figure 3.

loci (Figs 4, 6 and 7). Such a defect in GGR cannot be observed genetically as a UV-sensitivity phenotype of *rad26 hpr1/tho2* double mutants. In addition, in GGR-less strains the double mutations *rad26 hpr1/tho2* confer a more severe UV-sensitivity phenotype than each single mutation separately. These results indicate that the putative role that Hpr1 and Tho2 might have in NER is not exclusive of TCR and is different to that of Rad26. Indeed, this is not the only difference between Rad26 and Hpr1/Tho2, because in contrast to *hpr1* and *tho2* cells, *rad26* cells do not show a detectable hyper-recombination phenotype and are able to transcribe the bacterial *lacZ* sequences (S.González-Barrera, A.G.Rondón and A.Aguilera, unpublished results).

The negative effect of *rad7* on the accumulation of *RPB2* mRNA (Fig. 5) indicates that defective transcription does not necessarily imply defective TCR. However, it may not be surprising that the transcription elongation defect of *hpr1* and *tho2* is responsible for the TCR defect. The NER defects of *hpr1* and *tho2* cells are unlikely to be caused by lack of expression of a particular NER gene, because global transcription analysis of *tho2* cells using DNA microarrays shows that all known NER genes are transcribed (M.Gallardo, K.Ohta and A.Aguilera, unpublished results).

It is interesting to note that *hpr1* and *tho2* cells show a low rate of DNA repair, that is, a low amount of UV lesions repaired per minute as inferred by the slope of the curves of Figure 4. However, such slopes are similar in wild-type and *hpr1* and *tho2* mutants after 60–80 min upon UV irradiation (Fig. 4), suggesting that after this time, mutant cells recover the wild-type capacity of repair. In any case, the overall levels of removed UV lesions are still below wild-type levels at 120 min upon UV irradiation. This might reflect a process of adaptation. It is known that pre-irradiation of yeast cells enhances the

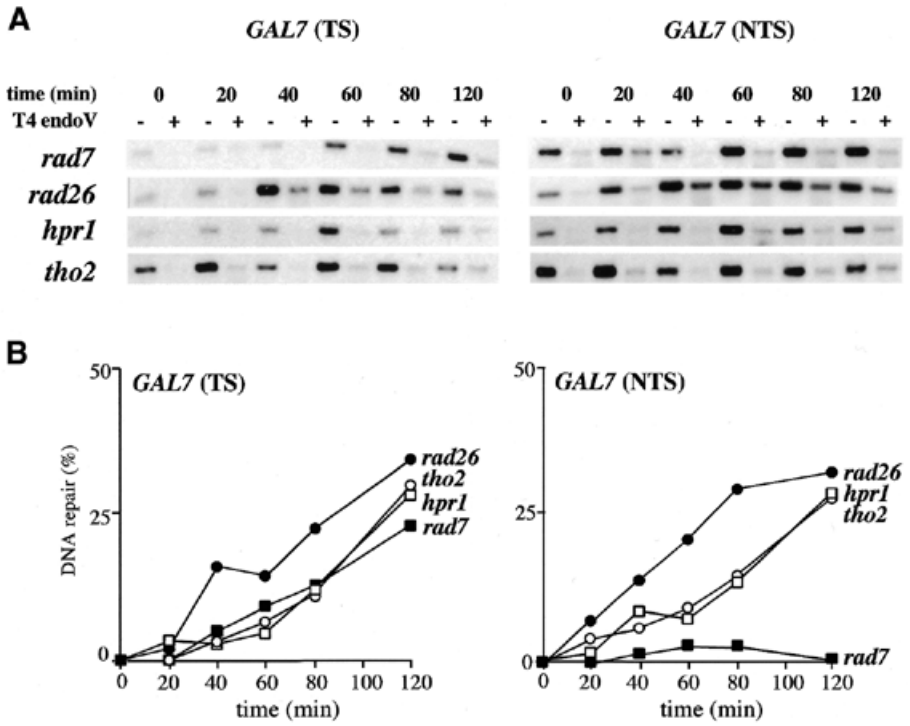

Figure 6. Repair analysis at the endogenous *GAL7* gene under repressed conditions, that is in cells grown in YEPD (2% glucose) cultures. (**A** and **B**) as in Figure 4. The experiments were repeated three times for each genotype, giving similar results with standard deviations below 20%. Only average values are shown.

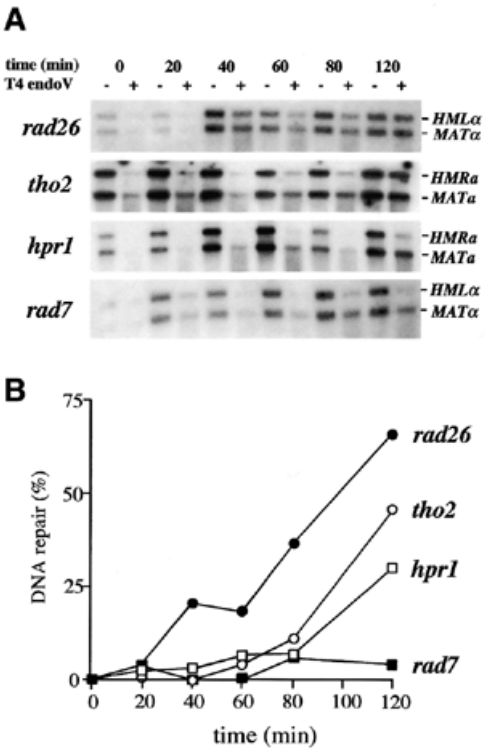

Figure 7. Physical analysis of DNA repair at the endogenous *HMLα* and *HMRa* silenced loci. (**A** and **B**) as in Figure 4. As the *HML* and *HMR* probes used also hybridize with the *MAT* locus, the corresponding *MAT* band is also detected. The experiments were repeated twice for each genotype, giving similar results with standard deviations below 20%. Only average values are shown.

removal of lesions induced by a second UV dose (1). Therefore, we cannot exclude the possibility that a slower adaptation response of *hpr1* and *tho2* cells to UV damage could make the DNA repair defects of *hpr1* and *tho2* mutants more severe.

At first, a transient sequestering of NER proteins could explain the NER defects of *hpr1* and *tho2* mutants. If the defects of *hpr1* and *tho2* in transcription elongation lead to frequent stalling of the elongating RNAPII, independently of UV lesions, a recruitment of the NER machinery could occur at many sites. As a consequence, NER proteins would be transiently sequestered, leading to a reduction of available NER components, and a concomitant defect in repair. Thus, a stalled RNAPII could recruit TFIIH and other NER factors regardless of the presence of a DNA lesion. Alternatively, the NER defects of *hpr1* and *tho2* could be explained by a putative unknown role of THO in NER.

Proteins connecting DNA repair and transcription could include, besides TFIIH, the transcription terminator factors 2 from *Drosophila* and humans (DmF2 and HuF2) and XAB2, a human protein identified by its ability to bind the NER protein XPA. DmF2 and HuF2 are members of the SWI2/SNF2 family (41,42) and dissociate RNAPI and RNPAII stalled at a CPD (43). XAB2 interacts with CSB, CSA and RNAPII and anti-XAB2 antibodies inhibit TCR and transcription when microinjected in fibroblasts (44). Interestingly, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* orthologs of *XAB2* seem to have a role in splicing (45,46), opening the possibility that RNA-processing proteins associated with elongation might influence TCR.

In this sense it is important to note that *hpr1* mutants are affected in RNA export (47) and that overexpression of Sub2, a putative RNA helicase involved in RNA splicing and export

(48,49), suppresses the hyper-recombination phenotype of *hpr1* cells (50). In addition, *sub2* mutants show similar hyper-recombination phenotypes to *hpr1* (50). Furthermore, a genetic interaction between Sub2 and Rad3, a component of TFIIH involved in RNAPII-dependent transcription and NER, has recently been inferred from the isolation of *rad3* alleles that suppress the growth defects of *sub2* (51). Our study, therefore, not only extends the possible functional role of THO to NER, but opens the possibility of a connection between DNA repair, transcription and the processing of the nascent RNA. The identification of the biochemical function of the THO complex will help to understand the mechanisms connecting these processes.

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