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Recovery of RNA polymerase II synthesis following DNA damage in mutants of *Saccharomyces cerevisiae* defective in nucleotide excision repair

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ABSTRACT

We have measured the kinetics of the recovery of mRNA synthesis in the inducible *GAL10* and *RNR3* genes after exposure of yeast cells to ultraviolet (UV) radiation. Such recovery is abolished in mutant strains defective in nucleotide excision repair (NER) of DNA, including a *rad23* mutant. Mutants defective in the *RAD7* or *RAD16* genes, which are required for the repair of the non-transcribed strand but not the transcribed strand of transcriptionally active genes, show slightly faster recovery of RNA synthesis than wild-type strains. A strain deleted of the *RAD26* gene, which is known to be required for strand-specific NER in yeast, manifested delayed recovery of mRNA synthesis, whereas a *rad28* mutant, which does not show defective strand-specific repair, showed normal kinetics of recovery. Measurement of the recovery of expression of selected individual yeast genes by Northern analysis following exposure of cells to UV radiation apparently correlates directly with the capacity of cells for strand-specific NER.

INTRODUCTION

A number of studies have demonstrated that the rate at which UV radiation-induced cyclobutane pyrimidine dimers (CPD) in RNA polymerase II (RNAP II)-transcribed genes varies as a function of their transcriptional activity. In yeast and human cells it has been demonstrated that CPD located in actively transcribed regions of the genome are repaired by nucleotide excision repair (NER) faster than lesions located in transcriptionally quiescent regions of the genome (1,2). In addition, in *Escherichia coli*, yeast and mammalian cells CPD in the transcribed strand of transcriptionally active genes are repaired faster than lesions in the non-transcribed strand (3–6). This phenomenon is referred to as transcription-coupled repair or strand-specific repair (7). There is evidence suggesting that other types of base damage, including those repaired by the base excision repair mode also exhibit a DNA strand bias, although the latter issue remains somewhat controversial (7).

The precise mechanism of strand-specific NER remains to be fully elucidated. Genes have been identified in *E.coli*, yeast and mammalian cells whose polypeptide products appear to be indispensable for strand-specific NER of CPD. *Escherichia coli* strains defective in the *mfd*$^+$ gene lose the ability to preferentially repair the transcribed strand of the *lacI*$^+$ gene following exposure of cells to UV radiation, and *mfd* mutant cells are moderately sensitive to UV radiation (8). Purified Mfd protein has been shown to displace RNA polymerase stalled at CPD sites during *in vitro* transcription (9). Additionally there are indications that Mfd protein can interact with UvrA protein and in this way may target the NER machinery to sites of base damage in transcribed strands at which *E.coli* RNA polymerase is stalled (9).

Cells from humans with the hereditary disorder Cockayne syndrome (CS) have a reduced ability to preferentially repair CPD in the transcribed strand of actively transcribed genes (10). Like *E.coli mfd* cells, cells from the human genetic complementation groups CS-A and CS-B are moderately UV sensitive (11). CS patients manifest a variety of disorders, including photosensitivity, profound growth defects and neurological abnormalities (11). However, it is not established that these phenotypes arise directly from defective strand-specific repair of DNA damage. The *CSA* gene encodes a polypeptide which is a member of a class of proteins called WD-repeat, WD-40 repeat or GH-WD proteins (12). Members of this class are involved in a variety of cellular processes (13). The *CSB* gene encodes a member of the SWI2/SNF2 family of nucleotide binding proteins, several of which have been shown to be DNA-dependent ATPases which perturb the conformation of chromatin in some way (14,15). Purified CSB protein is also a DNA-dependent ATPase (16).

Yeast homologs of the *CSA* and *CSB* genes have been identified by sequence homology and are designated *RAD28* (17) and *RAD26* (18), respectively. *RAD28* encodes a WD-repeat protein and *RAD26* encodes a member of the SWI2/SNF2 family with known DNA-dependent ATPase activity (19). Surprisingly, cells deleted of either or both the *RAD28* or *RAD26* genes are not abnormally sensitive to killing by UV radiation (17,18). Like human CS-B cells, yeast strains deleted of the *RAD26* gene manifest defective strand-specific repair of CPD in actively transcribed genes (18). However, in contrast to human CS-A cells, *rad28* mutant cells do not display this phenotype (17). The

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entire yeast genome has been sequenced and the *RAD28* gene is considered to be the unequivocal structural homolog of the human *CSA* gene (17). The lack of concordance between the phenotypes of human *CSA* and yeast *rad28* cells is therefore interesting.

Two other yeast genes have been identified with defects in strand-specific NER. Strains carrying mutations in the *RAD7* and *RAD16* genes exhibit intermediate sensitivity to UV radiation and show a defect in the ability to remove CPD from the non-transcribed strand of actively transcribed genes, and from both strands of transcriptionally silent genes (20). No human homologs of *RAD7* or *RAD16* have been identified.

It has been demonstrated that the majority of CS-A and CS-B cells manifest a significant delay in the recovery of total RNA synthesis following UV irradiation (21). Indeed, it was the discovery of this phenotype that first suggested a defect in transcription-dependent NER in CS cells (21). The experimental protocol used in these studies involves pulsing cells with a radiolabeled precursor for RNA synthesis, a technique which is believed to predominantly measure RNAP II transcription. However, it is not clear to what extent this procedure also measures RNAP I and III synthesis. The kinetics of the recovery of RNA synthesis following DNA damage in yeast have not been reported. Hence, systematic examination of this phenomenon might help elucidate the role of NER proteins in strand-specific repair of transcriptionally active genes, and the role of such repair in the resumption of RNAP II transcription following UV irradiation. Rather than examine total RNA synthesis following irradiation, we have developed an experimental protocol to examine the expression of transcripts from a single gene following irradiation in yeast cells. The yeast *Saccharomyces cerevisiae* has been well characterized with respect to genes required for or associated with NER (22). We have therefore used this protocol to examine the recovery of RNA synthesis in a number of yeast strains defective in NER. Additionally we have asked whether *rad26* and the *rad28* mutants mimic the delayed recovery of RNA synthesis observed in human CS-A and CS-B cells.

MATERIALS AND METHODS

Yeast strains

The yeast strains used in this study are listed in Table 1. All of the deletion strains were constructed using the one-step disruption technique (23). Strain SX46Δ*rad1*::*URA3* was generated by transformation of SX46 with *Pvu*II-digested plasmid pWS1510. This plasmid contains the blunt-ended *Pvu*II–*Hpa*I fragment of *RAD1* at the *Hin*cII site of a pUC19 derivative in which the *Hin*dIII site has been destroyed by filling in. The *RAD1* gene was gapped with *Hin*dIII and replaced with *URA3* on a *Hin*dIII fragment from YEp24. Strain SX46Δ*rad2*::*TRP1* has been described (24). Strain SX46Δ*rad7*::*HIS3* was constructed by transformation of SX46 with *Eco*RI-digested plasmid pΔ*rad7*::*HIS3* and was generously provided by Dr Simon H.Reed from our laboratory. This plasmid was constructed by digesting plasmid pΔ*rad7*::LEU2 (20) with *Kpn*I and *Hin*dIII, blunting the ends by filling in, and replacing the *LEU2* gene with a 1.8 kb *Bam*HI fragment containing *HIS3*. Strain SX46Δ*rad16*::*HIS3* was made by transformation of SX46 with *Eco*RI–*Bam*HI-digested plasmid pΔ*rad16*::*HIS3* and was generously provided by Dr Simon H.Reed from our laboratory. This plasmid was constructed by isolating the *RAD16* gene from pAS1-*RAD16* (25) on a *Sfi*I–*Bam*HI fragment in which the ends had been blunted by filling in, and cloning this fragment into *Sma*I-digested plasmid pUC18. The blunted *Bam*HI *HIS3* fragment was inserted into the *Eco*RV gapped *RAD16* gene. Strain SX46*rad23*::*HIS3* was constructed by transformation of SX46 with *Eco*RI–*Bam*HI-digested plasmid p*rad23*::*HIS3*. This plasmid was constructed by isolating the *RAD23* gene from plasmid pAS1-*RAD23* (25) on an *Nco*I–*Bam*HI fragment in which the ends had been blunted by filling in, and cloning this fragment into *Sma*I-digested plasmid pUC18. The blunted *Bam*HI *HIS3* fragment was inserted into the *Eco*RV site of the *RAD23* gene. SX46Δ*rad26::HIS3* was constructed by transformation of SX46 with *Sal*I/*Sna*BI linearized pTZΔ*rad26::HIS3* (18). SX46Δ*rad28*::*URA3* was constructed using the strategy described (17) and was generously provided by Dr William J.Feaver from our laboratory.

Table 1. Yeast strains

Strain	Genotype
SX46	***a*** *RAD ade2 his3-532 trp1-289 ura3-52*
SX46Δ*rad1::URA3*	***a*** *Δrad1::URA3 ade2 his3-532 trp1-289 ura3-52*
SX46Δ*rad2::TRP1*	***a*** *Δrad2::TRP1 ade2 his3-532 trp1-289 ura3-52*
SX46Δ*rad7::HIS3*	***a*** *Δrad7::HIS3 ade2 his3-532 trp1-289 ura3-52*
SX46Δ*rad16::HIS3*	***a*** *Δrad16::HIS3 ade2 his3-532 trp1-289 ura3-52*
SX46Δ*rad23::HIS3*	***a*** *Δrad23::HIS3 ade2 his3-532 trp1-289 ura3-52*
SX46Δ*rad26::HIS3*	***a*** *Δrad26::HIS3 ade2 his3-532 trp1-289 ura3-52*
SX46Δ*rad28::URA3*	***a*** *Δrad28::URA3 ade2 his3-532 trp1-289 ura3-52*

Recovery of RNA synthesis

Cells were grown in 100 ml YP Raffinose at 30°C to an OD_{600} of ~1. The cells were harvested by centrifugation and resuspended in 100 ml of phosphate buffered saline (pH 7) and irradiated with a 254 nm peak output germicidal lamp at a fluence rate of 1 J/m^2 for 70 s. Aliquots of cells were taken before and after irradiation to determine survival by plating on YP Galactose plates. Cells were harvested by centrifugation and resuspended in 100 ml YP Galactose medium and grown at 30°C. At the indicated time points 10 ml aliquots were taken, the cells collected by centrifugation, frozen in a dry ice/ethanol bath, and maintained at –80°C until RNA isolation.

Northern (RNA) analysis

Total RNA was isolated by the hot phenol technique as described (26). The RNA was fractionated on a 1% agarose gel containing 0.66 M formaldehyde and blotted onto Genescreen Plus as suggested by the manufacturer. Filters were probed with *GAL10* and *RNR3* DNA probes labeled by the random primer method. Hybridization was performed at 43°C in 5× SSPE, 50% formamide, 5× Denhardt's solution, 1% SDS, 10% dextran sulfate containing 100 μg/ml denatured salmon sperm DNA. The filters were washed twice in 2× SSPE at room temperature for 15 min, and twice in 2× SSPE, 2% SDS at 65°C for 45 min. Quantitation was performed on a PhosphorImager using Imagequant software and each data point represents the mean of at least three independent experiments in all the data shown. For most data points shown the standard error of the mean was <1%. Loading of RNA samples in different lanes did not differ significantly over the course of individual experiments.

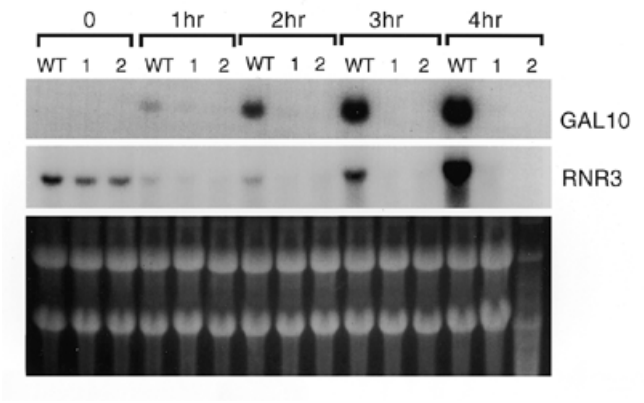

Figure 1. Recovery of RNA synthesis in wild-type versus *rad1* and *rad2* cells. The top two panels show hybridization of the indicated probes to the Northern blot. The bottom panel shows the ethidium bromide-stained gel prior to transfer. The time points indicate the hours following irradiation at which aliquots of cells were taken for preparation of RNA. Lanes WT are SX46 cells, lanes 1 are SX46Δ*rad1::URA3* and lanes 2 are SX46Δ*rad2::TRP1*.

RESULTS

Recovery of RNA synthesis following irradiation

The primary goal of this study was to quantitate the kinetics of the resumption of RNA synthesis following UV irradiation of yeast cells. We utilized Northern analysis to monitor the kinetics of the expression of single genes. In order to avoid potential interpretive complexities associated with the presence of RNAP II transcripts expressed both prior to and following exposure of cells to UV radiation, we initially utilized a target gene which is transcriptionally silent, but which can be induced following exposure of cells to UV light. The transcriptional regulation of the *GAL10* gene has been shown to be strictly dependent on the nutritional source of carbon (27). Essentially no transcription of the gene can be detected in the presence of raffinose. However, *GAL10* expression is fully induced within 30 min by transferring cells to medium containing galactose (27). In our experimental protocol cells were grown in raffinose medium, transferred to phosphate buffered saline, irradiated, and then transferred to galactose medium. Aliquots of cells were collected at intervals following irradiation and *GAL10* mRNA was detected by Northern blotting. In the absence of UV irradiation all of the strains examined showed normal induction of the *GAL10* gene within 30 min (data not shown).

Compared to unirradiated cells, UV-irradiated wild-type cells accumulated normal levels of *GAL10* transcripts only over the course of several hours (Fig. 1). Thus, the presence of photoproducts in DNA apparently delays the initiation, elongation and/or completion of *GAL10* transcription in these cells. In order to demonstrate that this delay reflects the time required to remove sites of base damage from the yeast genome by NER we carried out similar experiments with *rad1* and *rad2* mutant strains, both of which are completely defective in NER of both transcriptionally active and transcriptionally silent UV-irradiated DNA (28). As shown in Figure 1, we failed to observe expression of *GAL10* transcripts following exposure of *rad1* or *rad2* mutant cells to UV light.

Table 2. Survival following UV irradiation

Strain	Survival (%)[a]
SX46	15
SX46Δ*rad1::URA3*	<0.001
SX46Δ*rad2::TRP1*	<0.001
SX46Δ*rad7::HIS3*	0.03
SX46Δ*rad16::HID3*	0.007
SX46*rad23::HIS3*	0.029
SX46Δ*rad26::HIS3*	9
SX46Δ*rad28::URA3*	21

[a]Survival was determined as described in Materials and Methods. Cells were exposed to UV radiation using a 254 nm peak output germicidal lamp at a fluence rate of 1 J/m^2/s for 70 s. All measurements were performed in duplicate.

To demonstrate that this result was not unique to the *GAL10* gene we carried out similar studies with the DNA damage-inducible gene *RNR3*, which encodes one of the two alternative forms of the large subunit of ribonucleotide reductase (29). Transcription of the *RNR3* gene is strongly induced by UV irradiation (29). To determine the time course of induction of the *RNR3* gene the hybridization filters were stripped and reprobed for the *RNR3* transcript. Figure 1 shows the kinetics of accumulation of this transcript in irradiated wild-type, *rad1* and *rad2* cells. It has been previously reported that *RNR3* is not transcribed in unirradiated cells (29). However, under our experimental conditions transcription was reproducibly observed in irradiated cells not subjected to post-irradiation incubation (Fig. 1). This may reflect the specifics of our growth conditions. Interpretation of the experiments using the *RNR3* gene is potentially complicated by the anticipation that the intensity of the observed transcription signals represents the sum of *RNR3* transcripts present prior to UV irradiation of cells plus those produced following irradiation. Indeed, in wild-type cells we observed a decrease in *RNR3* mRNA at early times post-irradiation, presumably reflecting degradation of the existing pool of transcripts, followed by a progressive increase in the level of *RNR3* mRNA as a function of the post-irradiation incubation time (Fig. 1). In contrast, *RNR3* transcripts were degraded during the time course of the experiments in *rad1* and *rad2* mutant cells (Fig. 1). Collectively these results suggest that photoproducts in the yeast genome (which are not repaired by NER in *rad1* and *rad2* mutants), block RNAP II transcription initiation and/or elongation. Consistent with this suggestion, arrested RNAP II transcription by CPD has been demonstrated *in vitro* (30). Hence, apparently the *GAL10* and *RNR3* genes contains at least one RNAP II blocking lesion in the great majority of cells, as expected (31,32). In keeping with established results, survival of these mutants following irradiation was very low (Table 2).

Recovery of RNA synthesis in *rad26* and *rad28* mutant strains

In contrast to *rad1* and *rad2* mutants, which show no detectable recovery of RNA synthesis during the first 4 h of post-irradiation incubation, strains deleted of the *RAD26* gene (yeast *CSB* homolog) showed a significant delay in accumulating *GAL10*

transcripts compared to wild-type cells. (Fig. 2A and B). Cells deleted of the *RAD28* gene (yeast *CSA* homolog) accumulated *GAL10* mRNA with similar kinetics as wild-type cells (Fig. 2A and B). Similar results were observed when monitoring *RNR3* expression (Fig. 2A and C). The difference in the kinetics of recovery of *GAL10* and *RNR3* transcripts in *rad26* mutants compared to wild-type and *rad28* mutants is not due to reduced survival of *rad26* cells following UV irradiation, since the survival of all three strains was similar (Table 2). Remarkably, while the *rad26* mutant shows a delay in the recovery of RNAP II transcription and in strand-specific repair of the *RPB2* gene (18), this strain does not manifest detectably increased sensitivity to killing by UV light.

Recovery of RNA synthesis in *rad7* and *rad16* mutant strains

Strains deleted of the *RAD7* and *RAD16* genes are unable to repair CPD in the non-transcribed (coding) strand of actively transcribed genes (20). Such lesions in the coding strand do not block RNAP II transcription *in vitro* (30). We were therefore interested in investigating the effect of mutations in these genes on the expression of transcripts following irradiation. No delay in the recovery of transcription of the *GAL10* and *RNR3* genes was observed in the *rad7* and *rad16* mutant strains compared to the wild-type strain (Fig. 3A). Multiple experiments indicated that recovery in these mutants was in fact reproducibly slightly more rapid (Fig. 3B and C), despite the fact that as shown in Table 2, survival of each of these mutants was reduced several orders of magnitude relative to wild-type cells. These results confirm the notion that NER of the transcribed strand of actively transcribed genes has no requirement for the Rad7 and Rad16 proteins, and also provides direct *in vivo* evidence that photoproducts in the non-transcribed strand of such genes do not block RNAP II transcription.

Recovery of RNA synthesis in the *rad23* mutant strain

Like *rad7* and *rad16* mutants, cells with mutations in the *RAD23* gene exhibit an intermediate sensitivity to UV irradiation. There is controversy about the NER defect in *rad23* mutants. Several studies have indicated that there is no detectable incision of DNA and no detectable removal of CPD from either strand of both actively transcribed and non-transcribed genes (33,34). However, one study has indicated a residual capacity of *rad23* mutants to repair the transcribed strand of actively transcribed genes (35). This residual repair capacity was proposed to account for the greater survival in these mutants compared to mutants totally deficient in NER (35). We examined the ability of a *rad23* mutant to express RNAP II transcripts following irradiation. As shown in Figure 4, the *rad23* mutant strain is completely defective in the recovery of RNAP II transcription following irradiation. The results are identical to those observed with *rad1* and *rad2* mutants, which are known to be totally deficient in NER (28). As expected, survival of the *rad23* mutant in our hands was similar to that of the moderately sensitive *rad7* and *rad16* mutants (Table 2).

DISCUSSION

Rather than measure total RNA synthesis, which does not unambiguously distinguish between transcription catalyzed by

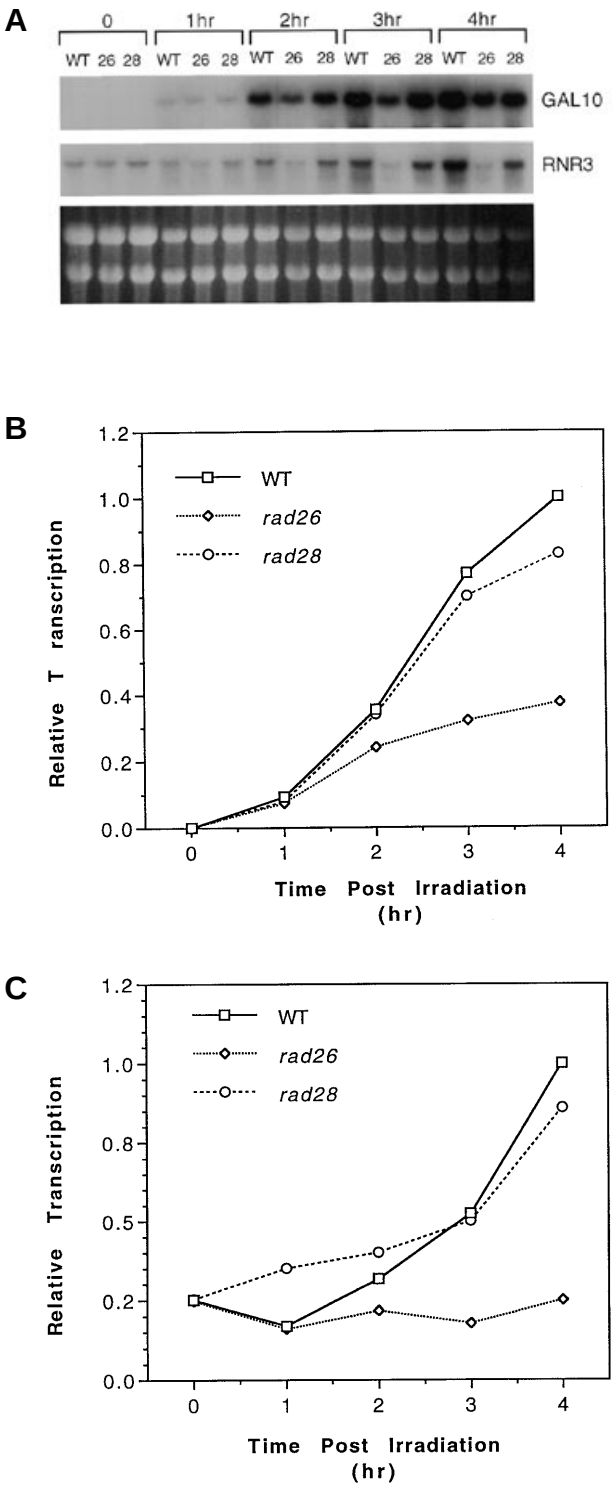

Figure 2. Recovery of RNA synthesis in wild-type versus *rad26* and *rad28* cells. (**A**) The top two panels show hybridization of the indicated probes to the Northern blot. The bottom panel shows the ethidium bromide-stained gel prior to transfer. The time points indicate the hours following irradiation at which aliquots of cells were taken for preparation of RNA. Lanes WT are SX46, lanes 26 are SX46Δ*rad26::HIS3* and lanes 28 are SX46Δ*rad28::URA3*. (**B**) Quantitation of the *GAL10* signal. The wild-type 4 h time point was normalized to 100% induction of transcription and all other values are shown relative to this. (**C**) Quantitation of the *RNR3* signal. The wild-type 4 h time point was normalized to 100% induction of transcription and all other values are shown relative to this.

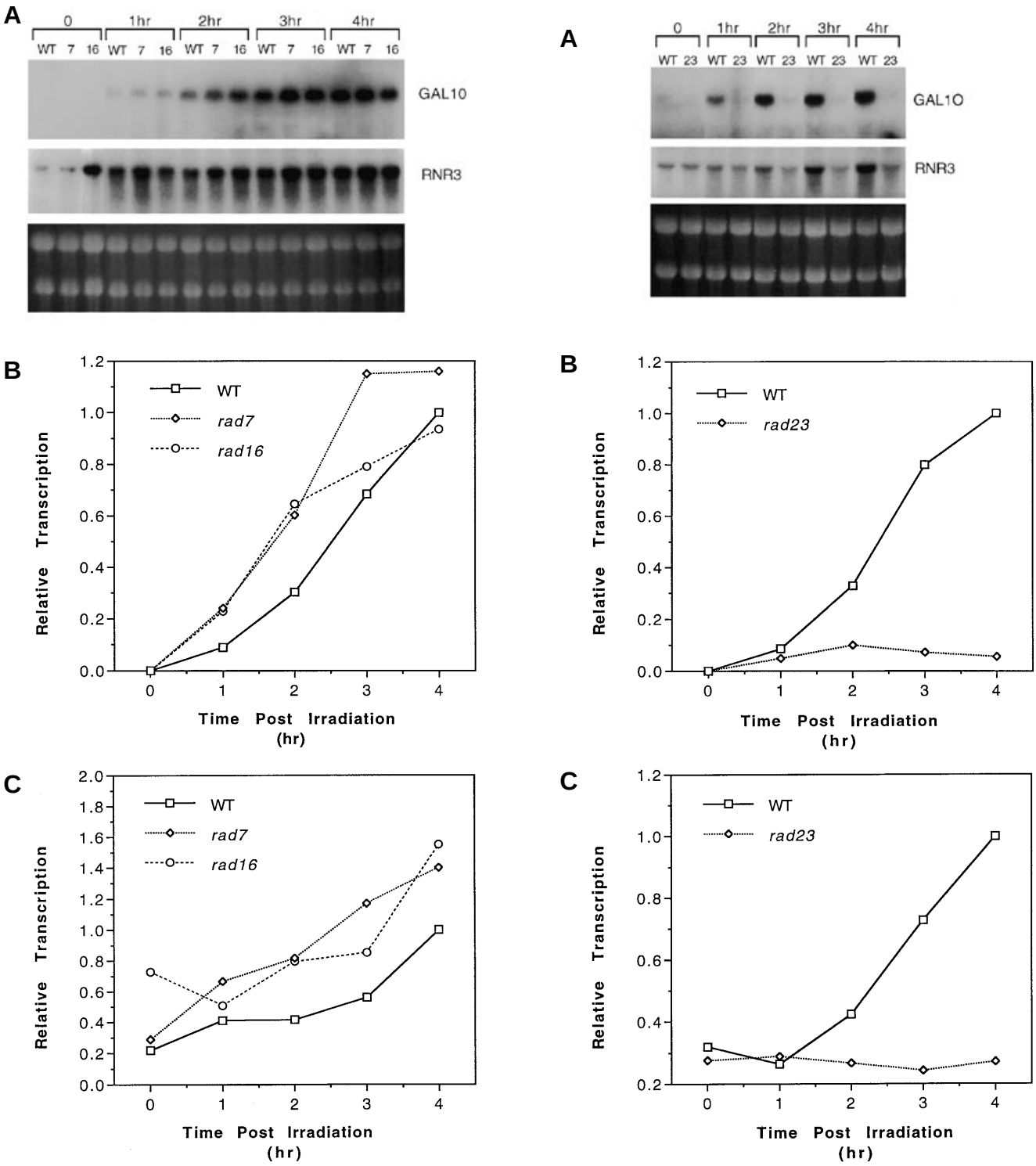

Figure 3. Recovery of RNA synthesis in wild-type versus *rad7* and *rad16* cells. (**A**) The top two panels show hybridization of the indicated probes to the Northern blot. The bottom panel shows the ethidium bromide-stained gel prior to transfer. The time points indicate hours following irradiation at which aliquots of cells were taken for preparation of RNA. The increased level of *RNR3* transcripts at zero time in the *rad16* mutant strain was not reproduced in several other experiments. Lanes WT are SX46, lanes 7 are SX46Δ*rad7::HIS3* and lanes 16 are SX46Δ*rad16::HIS3*. (**B**) Quantitation of the *GAL10* signal. The wild-type 4 h time point was normalized to 100% induction of transcription and all other values are shown relative to this. (**C**) Quantitation of the *RNR3* signal. The wild-type 4 h time point was normalized to 100% induction of transcription and all other values are shown relative to this.

Figure 4. Recovery of RNA synthesis in wild-type versus *rad23* cells. (**A**) The top two panels show hybridization of the indicated probes to the Northern blot. The bottom panel shows the ethidium bromide-stained gel prior to transfer. The time points indicate the hours following irradiation at which aliquots of cells were taken for preparation of RNA. Lanes WT are SX46, lanes 23 are SX46*rad23::HIS3*. (**B**) Quantitation of the *GAL10* signal. The wild-type 4 h time point was normalized to 100% induction of transcription and all other values are shown relative to this. (**C**) Quantitation of the *RNR3* signal. The wild-type 4 h time point was normalized to 100% induction of transcription and all other values are shown relative to this.

RNA polymerases I, II and III, we have developed a protocol by which we can measure the kinetics of recovery of RNA synthesis at the level of individual genes transcribed exclusively by RNAP II. This protocol should in principle be applicable to other regulated genes in yeast by employing appropriate inducing conditions following UV irradiation. Constitutively transcribed genes might also be amenable to this method if the transcripts are turned over very rapidly so that existing transcripts are largely degraded prior to recovery of RNA synthesis inhibited by DNA damage. However, constitutively transcribed genes that express stable transcripts are not well suited since the background of mRNA expressed prior to irradiation might complicate the accurate quantitation of recovery of mRNA synthesis following UV irradiation of cells.

We conclude that at the dose of UV radiation used for these experiments every *GAL10* and *RNR3* gene had at least one RNAP II blocking lesion in the transcribed strand (31,32). We therefore suggest that the kinetics of recovery of RNAP II synthesis in wild-type strains and in the various mutants examined, largely reflects the capacity of these strains to remove photoproducts from the transcribed strand of the *GAL10* and *RNR3* genes by NER. However, additional or alternative mechanisms involving the sequestration of transcription/NER factors (such as TFIIH) by DNA damage cannot be excluded at this time.

Mutants in the *RAD1* and *RAD2* genes, which are indispensable for NER of both transcriptionally silent and transcriptionally active DNA (28), are, as expected, totally defective in their ability to recover RNAP II transcription. A mutant deleted for the human homolog of the *CSB* gene, *RAD26*, which is defective in strand-specific NER but not in the repair of transcriptionally silent genes, manifests a substantial delay in the recovery of RNAP II transcription, while a mutant deleted for the human homolog of the *CSA* gene, *RAD28*, which is not defective in strand-specific repair, shows normal recovery of RNAP II transcription. As is the case with human *XPC* mutants (22), Yeast *rad7* and *rad16* mutants are specifically defective in NER of the non-transcribed strand of transcriptionally active genes (20). The kinetics of the removal of CPD from the transcribed strand of transcriptionally in *rad7* and *rad16* mutants has been reported to be indistinguishable from that in wild-type cells (20). In our experiments, these mutants reproducibly recovered RNAP II synthesis slightly more rapidly than wild-type cells. Conceivably the inability to carry out NER of the non-transcribed strand makes more NER complexes available for repair of the transcribed strand. Hence, measurement of the kinetics of recovery of RNAP II transcription after UV irradiation of cells may be a sensitive indicator of their capacity to repair UV radiation-induced base damage in the transcribed strand of transcriptionally active genes. In summary, the ability of wild-type and mutant yeast cells to recover RNAP II synthesis in individual genes as measured by Northern analysis directly mirrors their strand-specific repair capacity and conceivably may be employed as an alternative assay for measuring strand-specific repair of DNA in individual genes.

It remains unclear why the yeast *rad26* mutant, which, like human CS-B cells, is defective in strand-specific repair of UV radiation damage and in the recovery of blocked RNA synthesis, is not abnormally sensitive to killing by UV radiation (18). It is also puzzling that the *rad28* mutant strain, the yeast homolog of human CS-B cells, manifests neither abnormal UV sensitivity nor defective strand-specific repair (17). Recent experiments in our laboratory have demonstrated yet another phenotypic distinction between *rad26* and *rad28* mutants. We have observed that *in vitro* RNAP II transcription from a plasmid-borne yeast promoter is inhibited in the presence of a second plasmid bearing base damage (Z.You, W.J.Feaver and E.C.Friedberg, unpublished observations). This inhibition is relieved in extracts of *rad* mutants defective in NER *in vitro*. Surprisingly, inhibition of RNAP II transcription in the presence of DNA damage is also relieved in extracts of *rad26* (but not *rad28* mutants), even though *rad26* (and *rad28*) mutants are proficient in NER *in vitro* (Z.You, W.J.Feaver and E.C.Friedberg, unpublished observations). A detailed interpretation of these results will be presented elsewhere. The point relevant to the present discussion is that differences in the phenotypes of *rad26* and *rad28* mutants have emerged from a variety of different assays, all of which relate NER to RNAP II transcription. These observations suggest distinct roles for the Rad26 and Rad28 proteins, and also emphasize apparent differences in the function of the yeast Rad28 and human CSA proteins.

It remains to be established why the viability of a *rad23* mutant is greater than that observed in other *rad* mutant strains such as *rad1*, *rad2*, *rad3*, *rad4*, *rad10* and *rad14* following exposure to UV radiation. Regardless, our studies demonstrate that there is no direct correlation between the ability to recover RNAP II synthesis (which presumably measures repair of transcribed DNA strands) and resistance to killing by UV radiation. For example, *rad7* and *rad16* strains are capable of recovering RNAP II transcription at least as rapidly as wild-type cells, even though >99.9% of the cells are destined to die. These results suggest that other perturbations of DNA metabolism (such as DNA replication) may be more important for determining cellular lethality after exposure to UV radiation.

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