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Yeast *RAD26*, a Homolog of the Human *CSB* Gene, Functions Independently of Nucleotide Excision Repair and Base Excision Repair in Promoting Transcription through Damaged Bases

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***RAD26* in the yeast *Saccharomyces cerevisiae* is the counterpart of the human Cockayne syndrome group B (*CSB*) gene. Both *RAD26* and *CSB* act in the preferential repair of UV lesions on the transcribed strand, and in this process, they function together with the components of nucleotide excision repair (NER). Here, we examine the role of *RAD26* in the repair of DNA lesions induced upon treatment with the alkylating agent methyl methanesulfonate (MMS). MMS-induced DNA lesions include base damages such as 3-methyl adenine and 7-methyl guanine, and these lesions are removed in yeast by the alternate competing pathways of base excision repair (BER), which is initiated by the action of *MAG1*-encoded *N*-methyl purine DNA glycosylase, and NER. Interestingly, a synergistic increase in MMS sensitivity was observed in the *rad26*Δ strain upon inactivation of NER or BER, indicating that *RAD26* promotes the survival of MMS-treated cells by a mechanism that acts independently of either of these repair pathways. The galactose-inducible transcription of the *GAL2*, *GAL7*, and *GAL10* genes is reduced in MMS-treated *rad26*Δ cells and also in *mag1*Δ *rad14*Δ cells, whereas a very severe reduction in transcription occurs in MMS-treated *mag1*Δ *rad14*Δ *rad26*Δ cells. From these observations, we infer that *RAD26* plays a role in promoting transcription by RNA polymerase II through damaged bases. The implications of these observations are discussed in this paper.**

Cockayne syndrome (CS) in humans is characterized by severe growth retardation, with the outward appearance of cachectic dwarfism, and individuals with CS suffer from impaired neurological development and mental retardation. Mutations in the *CSA* and *CSB* genes account for ~90% of CS cases, and the mean age of death in these patients is ~12 years (13). Mutations in the *CSA* and *CSB* genes abolish preferential repair of UV-induced DNA lesions (27), a phenomenon known as transcription-coupled repair (TCR) (10). Because of the defect in the TCR of UV lesions, CS patients display mild sun sensitivity; however, they do not suffer from the high incidence of skin cancers characteristic of xeroderma pigmentosum patients.

The *Saccharomyces cerevisiae RAD26* gene is the counterpart to the human *CSB* gene, and its inactivation creates a defect in the TCR of UV lesions in yeast (26). The CSB and Rad26 proteins are members of the SWI2/SNF2 family of ATPases, and both these proteins have DNA-dependent ATPase activity (4, 21). The mechanism of the TCR of UV lesions is best understood in *Escherichia coli*, where the Mfd protein, which is a DNA-dependent ATPase, displaces RNA polymerase (Pol) stalled at the lesion site in a reaction dependent upon ATP hydrolysis. Subsequently, via its interaction with UvrA, Mfd recruits the UvrA-UvrB-UvrC enzyme complex to the lesion site (22). The CSB and Rad26 proteins resemble Mfd in their DNA-dependent ATPase activity, but how these eukaryotic proteins bring about TCR is not known.

For the repair of UV lesions in both yeast and humans, TCR represents one subpathway of the nucleotide excision repair (NER) of UV lesions; the other subpathway of global genomic repair promotes the repair of UV lesions from nontranscribed regions of the genome. Since both these subpathways require NER proteins, such as XPA, ERCC1, and XPF in humans and their respective counterparts Rad14, Rad10, and Rad1 in yeast, TCR of UV lesions is abolished in both yeast and humans in the absence of any of these or other essential NER proteins.

Recently, evidence of the preferential removal of the oxidative DNA lesions thymine glycol (TG) and 8-oxoguanine (8-oxoG) from the transcribed strands in human cells has been presented (3, 9). TCR of these lesions, however, is not affected in cells defective in NER, but TCR is defective in cells derived from CS patients with mutations in the *CSB*, *XPB*, *XPD*, or *XPG* gene. From these observations, CSB, XPG, and TFIIH (since the XPB and XPD DNA helicases are components of TFIIH) have been inferred to act in the displacement of RNA Pol II arrested at the sites of these damaged bases and subsequently to aid in the recruitment to the lesion sites of the components of base excision repair (BER), such as DNA glycosylases and apurinic endonucleases. Thus, in the TCR of UV lesions, CS proteins function with the NER proteins, but in the TCR of 8-oxoG- and TG-damaged bases, CS proteins have been proposed to act specifically in the recruitment of BER proteins.

Recent studies have indicated that *RAD26* plays a role in Pol II-dependent transcription elongation in yeast cells in the absence of any exogenous DNA damage (8) and that purified CSB protein increases the rate of elongation by Pol II on

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oligo(dC)-tailed DNA templates in the absence of any additional transcription factors (20). The involvement of Rad26/CSB proteins in transcription elongation through naturally occurring arrest sites raised the possibility that these proteins might also enable Pol II to transcribe through damaged bases. In that case, by freeing the lesion from stalled Pol II, the Rad26 and CSB proteins would promote the accessibility of DNA lesions to repair enzymes. In such a scenario, the Rad26 and CSB proteins would act independently of any of the repair processes.

Here, we use the yeast system to examine the role of *RAD26* in promoting transcription through DNA lesions induced by the alkylating agent methyl methanesulfonate (MMS). MMS alkylates bases in DNA, particularly adenine at the N3 position (3meA) and guanine at the N7 position (7meG). We show that transcription through such damaged bases is severely inhibited in *rad26*Δ cells lacking both the NER and BER pathways required for the removal of these lesions. Consequently, sensitivity to MMS is greatly enhanced in *rad26*Δ cells lacking NER, BER, or both of these repair mechanisms. These results implicate the Rad26 protein in the promotion of transcription through damaged bases, and they indicate that in this role Rad26 functions independently of these two DNA repair processes.

MATERIALS AND METHODS

Yeast strains. In this study, the wild-type strain EMY74.7 (*MAT***a** *his3-*Δ*1 leu2-3,112 trp1*Δ *ura3-52*) and its isogenic derivative strains YRP296, YR14.42, YR14.80, YR26.1, and YR14.82, which carry the *mag1*Δ, *rad14*Δ, *mag1*Δ *rad14*Δ, *rad26*Δ, and *mag1*Δ *rad14*Δ *rad26*Δ mutations, respectively, were used. The other strains used in this study, which were also derived from EMY74.7, were YR1.63, YR4.1, YR1.139, YR4.20, YR14.24, and YR26.25, and they carried the *rad1*Δ, *rad4*Δ, *rad1*Δ *rad26*Δ, *rad4*Δ *rad26*Δ, *rad14*Δ *rad26*Δ, and *mag1*Δ *rad26*Δ mutations, respectively.

MMS treatment. For the determination of survival rates after MMS treatment, cells were grown overnight in YEPD (yeast extract-peptone-dextrose) medium. The cells were washed with distilled water and resuspended in 0.05 mM KPO_4 at a density of 3×10^8 cells per ml. Aliquots (0.5 ml) of cells were treated with MMS at 30°C for 20 min with vigorous shaking. MMS was neutralized with 1 ml of 10% Na thiosulfate, and appropriate dilutions were plated on YEPD for viability determinations.

Transcription analyses. For the examination of *GAL2*, *GAL7*, and *GAL10* transcription, cells were grown at 30°C to log phase in YPL (1% yeast extract, 2% peptone, 3.7% lactate) medium. The cells were diluted to an optical density at 600 nm of 0.5 in YPL medium containing 2% galactose and 0.25% MMS. Samples were taken at selected time points after the cells were transferred to the medium containing galactose and MMS. The cells were pelleted and frozen quickly in crushed dry ice. Frozen cells were maintained at −80°C until RNA isolation.

Total RNA was isolated by the hot-phenol method (1) and fractionated by electrophoresis on 1.4% agarose–6% formaldehyde gels, followed by transfer to Hybond nylon membranes (Amersham). Each DNA probe was ^{32}P labeled by the Multiprime DNA-labeling system (Amersham). Hybridization was performed at 42°C in a solution containing 40% formamide, 5% dextran sulfate, 1% sodium dodecyl sulfate (SDS), 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, and 1 M KPO_4 containing 100 μg of denatured herring sperm DNA per ml. The blots were washed twice at room temperature with 2× SSC–0.1% SDS for 5 and 10 min, once with 0.5× SSC–0.1% SDS for 30 min at 50°C, and once with 0.1× SSC–0.1% SDS for 15 min at 50°C. Quantitation of mRNA levels was performed in a PhosphorImager with ImageQuant software.

RESULTS

RAD26 functions independently of NER and BER in restoring survival to MMS-treated yeast cells. For the preferential repair of UV lesions on the transcribed DNA strand, Rad26 functions together with components of the NER machinery. Consequently, introduction of the *rad26*Δ mutation into a strain with deletions of any of the genes indispensable for NER, such as *RAD14*, confers no additional increase in the UV sensitivity of the NER-defective mutant (28).

To determine if Rad26 functions similarly with the NER machinery for the repair of MMS-induced DNA lesions on the transcribed strand, we examined the MMS sensitivity of the NER-defective *rad1*Δ, *rad4*Δ, and *rad14*Δ mutants singly and in combination with the *rad26*Δ mutation. The Rad1 and Rad10 proteins form a DNA endonuclease which incises the damaged strand on the 5′ side of the lesion, while the Rad4-Rad23 complex and the Rad14 protein function at the lesion recognition step (14). As shown in Fig. 1A, sensitivity to MMS was enhanced to about the same extent in the *rad1*Δ, *rad4*Δ, *rad14*Δ, and *rad26*Δ strains. Interestingly, a synergistic increase in MMS sensitivity was observed when the *rad26*Δ mutation was combined with the *rad1*Δ, *rad4*Δ, or *rad14*Δ mutation. These observations suggest a role for *RAD26* in promoting the survival of MMS-treated cells by a mechanism that functions independently of NER.

The alkylated bases, such as 3meA and 7meG, formed in DNA by the action of MMS are removed by an *N*-methyl purine DNA glycosylase encoded in yeast by the *MAG1* gene. This gene was previously referred to as *MAG* (1a); however, in keeping with the nomenclature for yeast genes, we refer to it as *MAG1*. Deletion of *MAG1* confers a much higher degree of MMS sensitivity than does deletion of any of the NER genes (Fig. 1A), indicating a more prominent role for *MAG1* than for NER in the removal of MMS-induced DNA damage. A synergistic enhancement in MMS sensitivity occurs in the absence of both *MAG1* and *RAD14* (Fig. 1B), indicating that NER competes with Mag1-dependent BER for the removal of MMS-induced DNA lesions.

Next, we examined the effect of the *rad26*Δ mutation on the MMS sensitivity of the *mag1*Δ and *mag1*Δ *rad14*Δ strains. Compared to the *mag1*Δ and *rad26*Δ single mutant strains, the *mag1*Δ *rad26*Δ double mutant strain exhibits a synergistic enhancement in MMS sensitivity, and the *mag1*Δ *rad14*Δ *rad26*Δ triple mutant strain displays a higher level of MMS sensitivity than the *mag1*Δ *rad14*Δ, *mag1*Δ *rad26*Δ, or *rad14*Δ *rad26*Δ double mutant strain (Fig. 1B). The synergistic enhancement in MMS sensitivity in the *rad26*Δ strain upon the inactivation of NER or BER implies that *RAD26* promotes the survival of MMS-treated cells by a mechanism that acts independently of both of these repair processes.

Inhibition of transcription in MMS-treated *rad26*Δ cells. Genetic studies of yeast have indicated a role for *RAD26* in the promotion of transcription elongation by Pol II through intrinsic arrest sites in undamaged cells (8). Because of the independence of the function of *RAD26* from NER and BER in restoring survival to MMS-treated cells, we considered the possibility that Rad26 can also promote transcription through MMS-induced DNA lesions. In that case, rather than promoting coupling to either of these repair processes following the removal of stalled Pol II from the lesion, Rad26 would aid in the repair of the transcribed strand by preventing the inhibi-

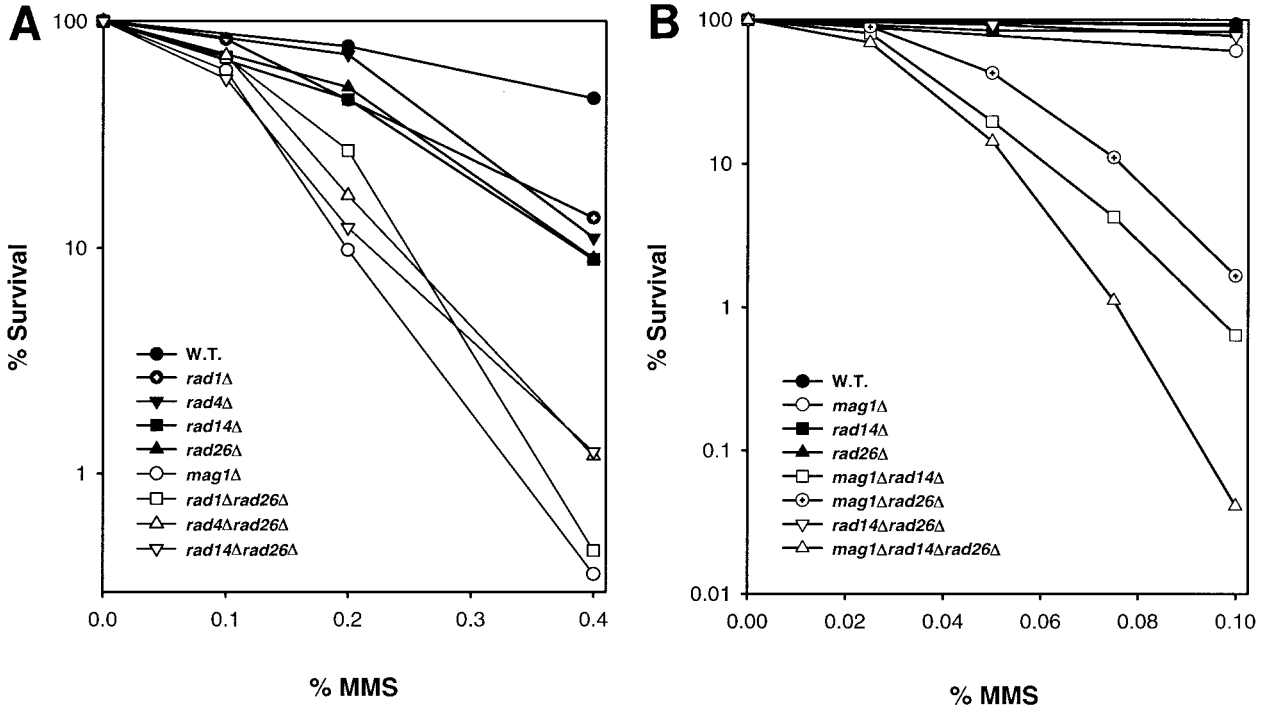


FIG. 1. Effect of the *rad26*Δ mutation on the survival of MMS-treated yeast cells lacking NER or BER. *RAD26* functions independently of NER (A) and *MAG1*-initiated BER (B) in promoting the survival of MMS-treated cells. The cells were treated with the indicated concentrations of MMS for 20 min at 30°C, and then MMS was inactivated with sodium thiosulfate and appropriate dilutions of cells were plated on YEPD for viability determinations. A concentration of 0.1% MMS corresponds to a 11.8 mM concentration of the chemical. The survival curves represent results from an average of three or more experiments for each strain. W.T., wild type.

tory effect of stalled Pol II on the ability of repair enzymes to gain access to the lesion.

For the TCR of UV-induced DNA lesions, *RAD26* functions with the NER machinery, where it may act in a manner analogous to that of the *E. coli* Mfd protein. In this role, following the displacement of stalled Pol II from the lesion site, Rad26 may promote the recruitment of the NER enzyme complex to the lesion site. For UV lesions, then, we expect transcription to remain inhibited in the absence of any of the essential NER protein factors, since in spite of the displacement of stalled Pol II from the lesion site, in the absence of any lesion removal, Pol II would continue to stall at the lesion site because of the persistence of such lesions. In agreement with this hypothesis, recovery of mRNA synthesis in the inducible *GAL10* and *RNR3* genes is abolished in UV-irradiated cells from NER-defective mutants whereas recovery of *RNR3* mRNA synthesis is abolished, and that of *GAL10* mRNA is very considerably slowed, in the UV-irradiated *rad26*Δ strain (15). In contrast, if Rad26 enabled Pol II to transcribe through MMS-induced DNA lesions, then we would expect a considerable level of transcription to persist even in the absence of both NER and BER. Transcription should decline precipitously, however, in the *rad26*Δ strain lacking both NER and BER.

To investigate the role of *RAD26* in promoting transcription through MMS-induced DNA lesions, we examined the synthesis of *GAL2*, *GAL7*, and *GAL10* mRNAs in the wild-type and *rad14*Δ, *mag1*Δ, *mag1*Δ *rad14*Δ, *rad26*Δ, and *mag1*Δ *rad14*Δ *rad26*Δ strains in MMS-treated and untreated cells. In the absence of MMS treatment, transcription of these *GAL* genes, which is induced upon the addition of galactose, was not reduced in the *rad14*Δ, *mag1*Δ, and *mag1*Δ *rad14*Δ mutant strains compared to that in the wild-type strain (Fig. 2). The levels of these transcripts, however, were lower in the *rad26*Δ strain than in the wild-type or *rad14*Δ, *mag1*Δ, and *mag1*Δ *rad14*Δ mutant strains, and the pattern of transcript accumulation in the *mag1*Δ *rad14*Δ *rad26*Δ strain was virtually identical to that in the *rad26*Δ strain (Fig. 2). The lack of any inhibition of transcription in the *mag1*Δ *rad14*Δ cells and the absence of any further inhibition of transcription in *mag1*Δ *rad14*Δ *rad26*Δ cells beyond the level of that in the *rad26*Δ cells suggest that there is no significant accumulation of lesions such as 3meA and 7meG in cells not treated with MMS.

In MMS-treated cells, however, the levels of these *GAL* gene transcripts were consistently lower in the *mag1*Δ *rad14*Δ strain than in the wild-type strain or the *rad14*Δ or *mag1*Δ single mutant strain (Fig. 3). This suggests that under the experimental conditions used the number of DNA lesions is such that Rad26 becomes limiting and is unable to promote transcription through all of the lesion sites in the *mag1*Δ *rad14*Δ strain. Importantly, the levels of all three transcripts were much lower in MMS-treated *rad26*Δ cells than in similarly treated *mag1*Δ *rad14*Δ cells, and a very severe reduction in transcription occurred in the *mag1*Δ *rad14*Δ *rad26*Δ strain (Fig. 3).

We compared the levels of *GAL2*, *GAL7*, and *GAL10* transcripts (Tables 1, 2, and 3, respectively) in MMS-treated and untreated mutant cells to those in similarly treated wild-type cells. While there is little or no evidence of a reduction in the

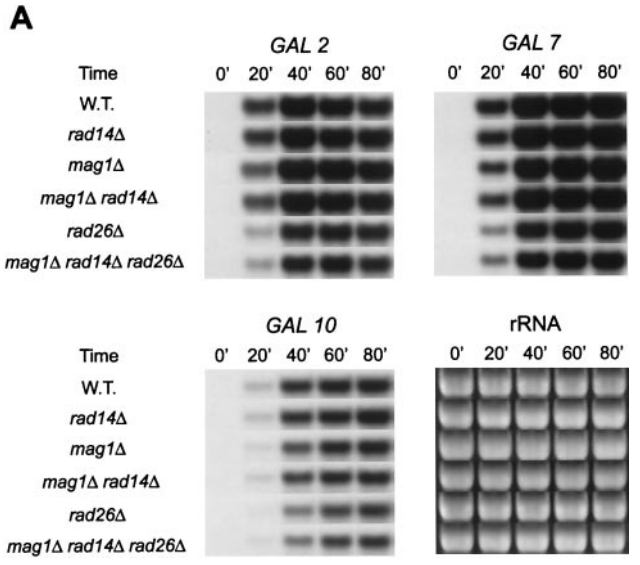
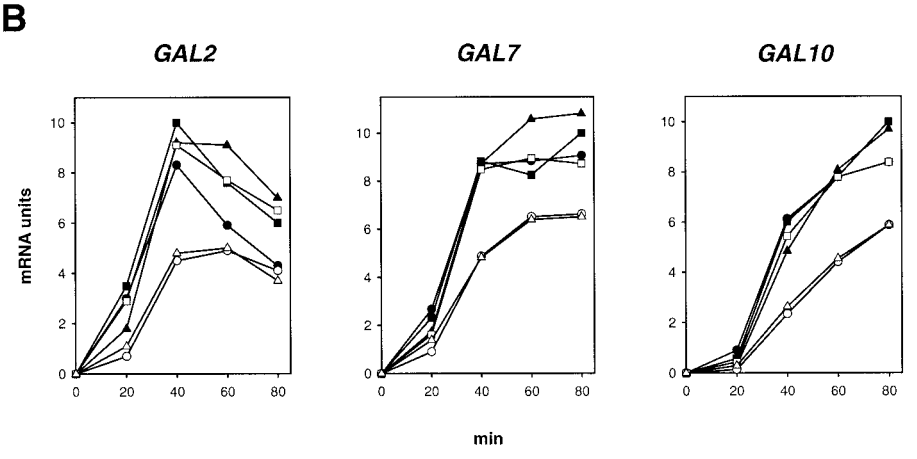


FIG. 2. Transcription of *GAL2*, *GAL7*, and *GAL10* genes in wild-type (W.T.) and mutant strains in the absence of MMS treatment. Total RNAs from cells grown in YPL medium containing galactose were subjected to Northern analyses. (A) Transcript levels of *GAL2* (top left panel), *GAL7* (top right panel), and *GAL10* (bottom left panel) genes. The ethidium bromide-stained gel (bottom right panel) indicates the levels of RNAs loaded. mRNA levels were examined at the indicated time periods after the cells were transferred to galactose-containing medium. (B) Quantitation of *GAL2*, *GAL7*, and *GAL10* mRNA levels. mRNA units at each time point are relative to the highest mRNA level in the *rad14*Δ strain. Symbols: ●, wild-type strain; ■, *rad14*Δ strain; ▲, *mag1*Δ strain; ○, *rad26*Δ strain; □, *mag1*Δ *rad14*Δ strain; △, *mag1*Δ *rad14*Δ *rad26*Δ strain.

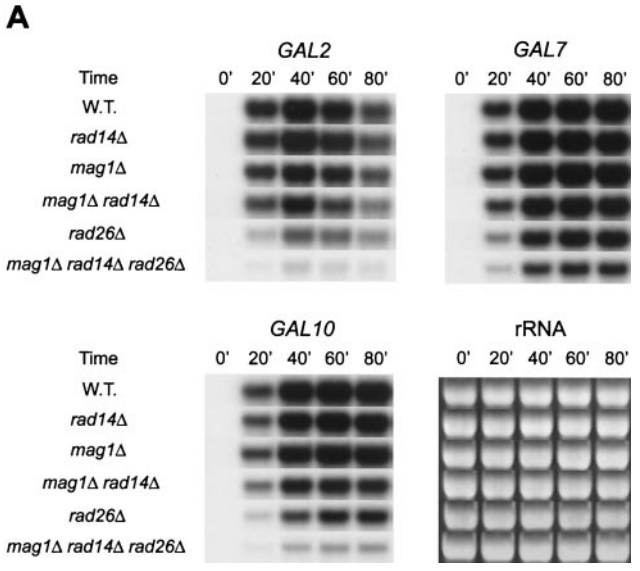


levels of these transcripts in MMS-treated *rad14*Δ and *mag1*Δ cells, the levels of transcripts of all three *GAL* genes were consistently lower in the MMS-treated *mag1*Δ *rad14*Δ strain than in the wild-type or the *rad14*Δ and *mag1*Δ mutant strains that were treated similarly. The levels of *GAL* gene transcripts were reduced even more in *rad26*Δ cells treated with MMS than in similarly treated *mag1*Δ *rad14*Δ cells, and a much more severe inhibition of transcription occurred in the *mag1*Δ *rad14*Δ *rad26*Δ strain than in the *mag1*Δ *rad14*Δ or *rad26*Δ strain.

DISCUSSION

***RAD26* promotes survival of MMS-damaged cells by a process that functions independently of NER or BER.** *RAD26* functions in the preferential repair of UV lesions on the transcribed strand in conjunction with NER, and consequently, introduction of the *rad26*Δ mutation into any of the NER-defective mutants, for example, the *rad14*Δ strain, causes no further increase in UV sensitivity. Here, we show that a synergistic increase in MMS sensitivity occurs in the *rad14*Δ *rad26*Δ double mutant strain compared to that in the *rad14*Δ or *rad26*Δ single mutant strain. A synergistic enhancement in MMS sensitivity also occurs upon the incorporation of the *rad26*Δ mutation into the *mag1*Δ strain. A synergistic rise in MMS sensitivity is also seen in the *mag1*Δ *rad14*Δ strain compared to that that in the *mag1*Δ and *rad14*Δ strains, and the *mag1*Δ *rad14*Δ *rad26*Δ strain displays a higher level of MMS sensitivity than any of the double mutants carrying any two of these three deletion mutations. From these observations, we infer that the NER and BER systems compete for the repair of MMS-induced DNA lesions and that Rad26 promotes the survival of MMS-damaged cells by a mechanism that acts independently of either of these repair processes.

RAD26 promotes transcription through damaged bases. The levels of *GAL* gene transcripts are reduced in MMS-treated *rad26*Δ cells, and a reduction in transcript levels also occurs in MMS-treated *mag1*Δ *rad14*Δ double mutant strains. Further, and importantly, a more precipitous decline in transcription occurs in the MMS-treated *mag1*Δ *rad14*Δ *rad26*Δ strain than in the similarly treated *mag1*Δ *rad14*Δ or *rad26*Δ strain. The accentuation of transcriptional defects that occurs in the *mag1*Δ *rad14*Δ strain upon the inactivation of *RAD26* implies that Rad26 enables Pol II to resume transcription



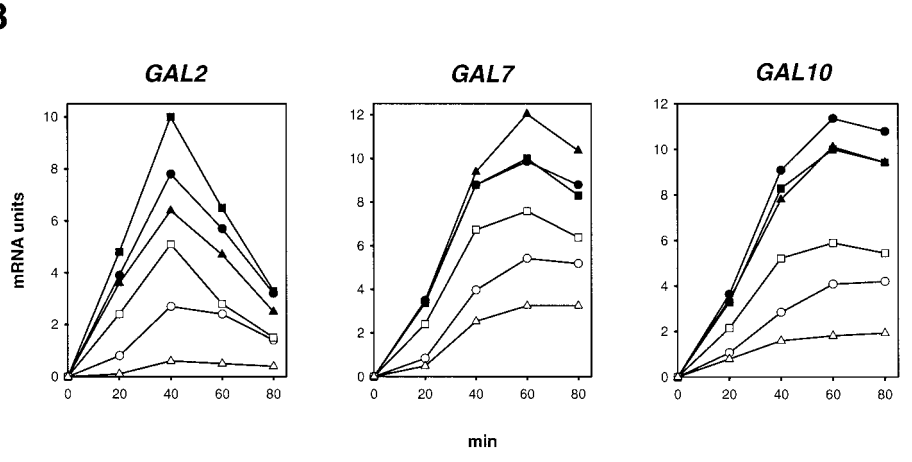


FIG. 3. Transcription of *GAL2*, *GAL7*, and *GAL10* genes in wild-type (W.T.) and mutant strains in the presence of MMS treatment. Total RNAs from cells grown in YPL medium containing galactose and MMS were subjected to Northern analyses. (A) Transcript levels of *GAL2* (top left panel), *GAL7* (top right panel), and *GAL10* (bottom left panel) genes. The ethidium bromide-stained gel (bottom right panel) indicates the levels of RNAs loaded. mRNA levels were examined at the indicated time periods after the cells were transferred to medium containing galactose and MMS. (B) Quantitation of *GAL2*, *GAL7*, and *GAL10* mRNA levels. mRNA units at each time point are relative to the highest mRNA level in the *rad14*Δ strain. Symbols: ●, wild-type strain; ■, *rad14*Δ strain; ▲, *mag1*Δ strain; ○, *rad26*Δ strain; □, *mag1*Δ *rad14*Δ strain; △, *mag1*Δ *rad14*Δ *rad26*Δ strain.

through MMS-induced DNA lesions in the absence of both NER and BER, the two repair systems available for the removal of these lesions. If Rad26 had promoted transcription by displacing stalled Pol II from the lesion site followed by the recruitment of the NER or BER proteins, then there should have been no further decline in the level of transcription in the *mag1*Δ *rad14*Δ *rad26*Δ strain beyond that seen in the *mag1*Δ *rad14*Δ strain. This is because, in the absence of both the repair systems, Pol II would stall at the site of the DNA lesions, and even if Pol II were to be removed from the lesion site by the action of Rad26, due to the persistent stalling of Pol II, this process would continue to yield incompletely formed transcripts which would then be subject to degradation. On the other hand, if Rad26 were to promote transcription through MMS-induced DNA lesions, then even in the absence of any removal of these lesions, full-length transcripts would be formed.

All our observations—the reduction in the levels of transcription in the *rad26*Δ and *mag1*Δ *rad14*Δ strains and the even greater reduction in the level of transcription in the *mag1*Δ *rad14*Δ *rad26*Δ strain—are in accord with the inference that Rad26 plays a role in the promotion of transcription through MMS-induced DNA lesions. Transcription is affected to a lesser degree in the *rad26*Δ or *mag1*Δ *rad14*Δ strain than in the *mag1*Δ *rad14*Δ *rad26*Δ strain, because lesions can still be removed by the NER and BER systems in the *rad26*Δ strain and

TABLE 1. Levels of *GAL2* mRNA in *rad14*Δ, *mag1*Δ, *mag1*Δ *rad14*Δ, *rad26*Δ, and *mag1*Δ *rad14*Δ *rad26*Δ mutant strains in the absence and presence of MMS

Time (min)[b]	*GAL2* mRNA level in strain with indicated deletion[a]									
	*rad14*Δ		*mag1*Δ		*mag1*Δ *rad14*Δ		*rad26*Δ		*mag1*Δ *rad14*Δ *rad26*Δ	
	−	+	−	+	−	+	−	+	−	+
0	0	0	0	0	0	0	0	0	0	0
20	117	123	60	92	97	62	23	21	37	2.6
40	120	128	110	82	110	65	54	35	58	7.7
60	129	114	154	82	131	49	83	42	85	8.8
80	140	103	163	78	151	47	95	44	86	12.5

[a] mRNA levels in the mutant strains are given relative to the mRNA levels in the wild-type strain times 100. −, MMS absent; +, MMS present.
[b] Time point of sample removal.

TABLE 2. Levels of *GAL7* mRNA in *rad14*Δ, *mag1*Δ, *mag1*Δ *rad14*Δ, *rad26*Δ, and *mag1*Δ *rad14*Δ *rad26*Δ mutant strains in the absence and presence of MMS

Time (min)[b]	*GAL7* mRNA level in strain with indicated deletion[a]									
	*rad14*Δ		*mag1*Δ		*mag1*Δ *rad14*Δ		*rad26*Δ		*mag1*Δ *rad14*Δ *rad26*Δ	
	−	+	−	+	−	+	−	+	−	+
0	0	0	0	0	0	0	0	0	0	0
20	86	97	65	100	61	69	30	24	52	14
40	101	100	100	107	97	77	56	45	55	29
60	93	101	120	122	101	77	74	55	73	33
80	110	95	119	118	96	73	73	59	72	37

[a] mRNA levels in the mutant strains are given relative to the mRNA levels in the wild-type strain times 100. −, MMS absent; +, MMS present.
[b] Time point of sample removal.

Rad26 can still promote the passage of Pol II through lesions in the *mag1*Δ *rad14*Δ strain, although these lesions cannot be removed. In the *mag1*Δ *rad14*Δ *rad26*Δ triple mutant strain, however, DNA lesions persist and transcription will continue to stall at these lesion sites.

Lesion bypass by Pol II. Transcription by RNA polymerases is not as accurate as DNA synthesis by replicative polymerases. In contrast to the high fidelity of nucleotide incorporation by the prokaryotic and eukaryotic replicative DNA polymerases, RNA polymerases incorporate an incorrect nucleotide with frequencies of 10^{-3} to 10^{-5} (24). Also, in contrast to the replicative DNA polymerase, *E. coli* RNA polymerase can carry out efficient bypass of lesions such as O^6-methyl guanine (m6G), 8-oxoG, and abasic sites located on the transcribed strand of a duplex DNA, and it incorporates a uracil opposite m6G, either an adenine or cytosine opposite 8-oxoG, and an adenine opposite abasic sites (29, 30). T7 RNA polymerase also transcribes through an 8-oxoG lesion present on the template strand by incorporating an adenine or cytosine opposite the lesion (2). In contrast to the efficient bypass of various damaged bases, *E. coli* RNA polymerase stalls at cyclobutane pyrimidine dimers (CPD) and has to be displaced by the Mfd protein, which subsequently recruits the NER proteins to the dimer site (22). A CPD, then, is more inhibitory to *E. coli* RNA polymerase than are the various base damages.

TABLE 3. Levels of *GAL10* mRNA in *rad14*Δ, *mag1*Δ, *mag1*Δ *rad14*Δ, *rad26*Δ, and *mag1*Δ *rad14*Δ *rad26*Δ mutant strains in the absence and presence of MMS

Time (min)[b]	*GAL10* mRNA level in strain with indicated deletion[a]									
	*rad14*Δ		*mag1*Δ		*mag1*Δ *rad14*Δ		*rad26*Δ		*mag1*Δ *rad14*Δ *rad26*Δ	
	−	+	−	+	−	+	−	+	−	+
0	0	0	0	0	0	0	0	0	0	0
20	67	91	33	94	50	59	17	29	33	22
40	98	91	79	86	88	58	38	31	43	18
60	100	88	104	89	100	52	57	36	58	16
80	119	87	116	87	100	51	70	39	70	18

[a] mRNA levels in the mutant strains are given relative to the mRNA levels in the wild-type strain times 100. −, MMS absent; +, MMS present.
[b] Time point of sample removal.

Like the prokaryotic RNA polymerases, eukaryotic Pol II is also unable to transcribe through CPDs, and it stalls at such lesion sites (19). Similar to Mfd in *E. coli*, the Rad26 and CSB proteins may be crucial for the removal of stalled Pol II from dimer sites. However, the Rad26 and CSB factors may enable Pol II to transcribe through various base damages, including oxidative DNA lesions such as 8-oxoG and TG, and such transcriptional lesion bypass may additionally require TFIIH and XPG.

Other considerations. NER is the primary mechanism available in eukaryotes for the repair of UV-induced DNA lesions, such as CPDs and (6-4) photoproducts, and for the removal of other distorting DNA lesions, such as intrastrand and interstrand cross-links and bulky adducts formed upon treatment with various chemical agents (14, 17). NER, however, also functions in the removal of a variety of damaged bases, where it competes with a diverse array of BER pathways. In vitro studies with human cell extracts have indicated that NER can remove a variety of damaged bases, including N^6-methyladenine, m6G, 8-oxoG, and TG, and abasic sites (5, 16). Genetic studies of yeast have provided in vivo evidence of the involvement of NER in the removal of abasic sites (23) and 8-oxoG lesions (18), and the genetic evidence presented here supports the hypothesis that NER plays a role in the removal of damaged bases such as 3meA and 7meG.

Consistent with the involvement of NER in the removal of a variety of damaged bases and abasic sites, xeroderma pigmentosum patients exhibit an increase in the frequency of internal cancers, particularly those in the brain and other parts of the central nervous system (6, 7). Our observation that Rad26 functions independently of NER or BER in promoting transcription through damaged bases suggests that the developmental problems of CS patients would worsen in the absence of NER. Although there are no known cases of such double mutations in humans, mice lacking both the *XPA* and *CSB* genes display severe growth retardation, ataxia, and motor dysfunction during early postnatal development, and they die before weaning (11). In contrast, *XPA*-deficient mice do not show any obvious developmental or neurological abnormalities (12), and *CSB*-deficient mice exhibit only mild growth and neurological abnormalities (25). Our findings for yeast provide an explanation for the more severe developmental and neurological defects seen in $XPA^{-/-}$ $CSB^{-/-}$ mice than in mice with single mutations, as they predict that in the absence of CSB, transcription would stall at the sites of naturally occurring base damages and at abasic sites that would accumulate in DNA in the absence of *XPA*. The stalling of transcription at such lesion sites would then be the cause of developmental defects in $XPA^{-/-}$ $CSB^{-/-}$ mice.

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