The Stalling of Transcription at Abasic Sites Is Highly Mutagenic

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Abasic (AP) sites represent one of the most frequently formed lesions in DNA. Here, we examine the consequences of the stalling of RNA polymerase II at AP sites in DNA in *Saccharomyces cerevisiae*. A severe inhibition of transcription occurs in strains that are defective in the removal of AP sites and that also lack the *RAD26* gene, a homolog of the human Cockayne syndrome group B (*CSB*) gene, and, importantly, a dramatic rise in mutagenesis is incurred in such strains. From the various observations presented here, we infer that the stalling of transcription at AP sites is highly mutagenic.

Cellular DNA is subjected to damage inflicted by external environmental agents such as UV light from the sun and chemical pollutants, by endogenous factors such as reactive oxygen species, and by spontaneous decay. Such damage to DNA is removed by nucleotide excision repair (NER) or base excision repair (BER) processes. However, lesions that escape repair can be a block to replication and transcription. In contrast to the information available for the pathways controlling the replication of damaged DNA (19), little is known about the processes that promote transcription through DNA lesions in eukaryotes, and also, meager information is available about the consequences of the stalling of transcription at DNA lesion sites. Here we examine the effects of abasic (AP) sites on RNA polymerase II (Pol II) transcription in *Saccharomyces cerevisiae* and determine if the stalling of transcription at AP sites has mutagenic consequences. AP sites are formed in DNA because of spontaneous hydrolysis of the N-glycosylic bond and as intermediates in BER processes by the action of DNA glycosylases following the release of damaged bases (23).

RAD26 is the *S. cerevisiae* counterpart of the human *CSB* gene. Mutations in *CSB* cause Cockayne syndrome (CS), which is characterized by severe growth retardation, progressive neurological dysfunction, mental retardation, and early death (12). Also, mutations in the *CSB* and *RAD26* genes confer a defect in preferential repair of UV lesions from the transcribed strand (21, 22), a phenomenon known as transcription-coupled repair (TCR) (11). The proteins encoded by the *CSB* and *RAD26* genes both possess a DNA-dependent ATPase activity (3, 17). Although the mechanism of TCR in yeast or humans is not known and there is no evidence that CSB disrupts the ternary complex of Pol II stalled at a lesion site (4, 15), studies with the *Escherichia coli* Mfd protein, which functions in TCR by displacing the stalled RNA polymerase from the thymine dimer in an ATP hydrolysis-dependent manner and which subsequently recruits the excision nuclease (18), have suggested that Rad26 (CSB) may act in an analogous manner in TCR of

UV-induced lesions. Also, a role for CSB in transcription elongation has been indicated by in vitro studies (16) and genetic studies of *S. cerevisiae* have provided evidence consistent with such a role for the Rad26 protein in vivo (10).

In *S. cerevisiae*, two class II AP endonucleases, encoded by the *APN1* and *APN2* genes, function in the removal of AP sites and NER comprises the third competing pathway for AP site removal (8, 20). Here we show that AP sites that accumulate in DNA in the absence of *APN1*, *APN2*, and NER block transcription and that this transcriptional block becomes more pronounced in the absence of *RAD26*. We also found that mutations occur at a very high rate in the *rad26Δ* mutant strain lacking the various pathways for the removal of AP sites. From these observations, we infer that the stalling of transcription at AP sites is extremely mutagenic.

MATERIALS AND METHODS

Strains and media. All of the deletion strains used in this study were derived from wild-type strain EMY74.7 (*MAT*a *his3-Δ1 leu2-3,112 trp1Δ ura3-52*). The generation of deletions of the *APN1*, *APN2*, *RAD14*, *REV3* (8, 20), *RAD26* (10), and *POL32* (5) genes by the one-step gene disruption method has been described previously.

MMS treatment. Cells were grown overnight in YPD (yeast extract-peptone-dextrose) medium for the determination of survival after treatment with methyl methanesulfonate (MMS). Cells were washed with distilled water and resuspended in 0.05 mM KPO_4 (pH 7.0) at a density of 3×10^8/ml. After treatment of aliquots of cells with MMS at 30°C for 20 min with vigorous shaking, MMS was neutralized with 10% Na thiosulfate. Appropriate dilutions were plated on YPD for viability determinations and on synthetic complete medium lacking arginine but containing canavanine for the identification of canavanine-resistant colonies.

Transcription analysis. For examination of *GAL7* and *GAL10* transcription, cells grown at 30°C to log phase in YPL (1% yeast extract, 2% peptone, 3.7% lactate) medium 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 the indicated time points after being transferred to galactose- and MMS-containing medium, at which time they 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 (10), and quantitation of mRNA levels was performed in a PhosphorImager with ImageQuant software.

Determination of spontaneous *CAN1*^{S}-to-*can1*^{r} mutation rates. For each strain, 11 independent cultures, each starting from ~10 canavanine-sensitive cells, were grown in 0.5 ml of YPD medium. Rates of forward mutations at the *CAN1* locus were determined from the number of canavanine-resistant colonies by the method of the median (9). Three experiments were performed with each strain.

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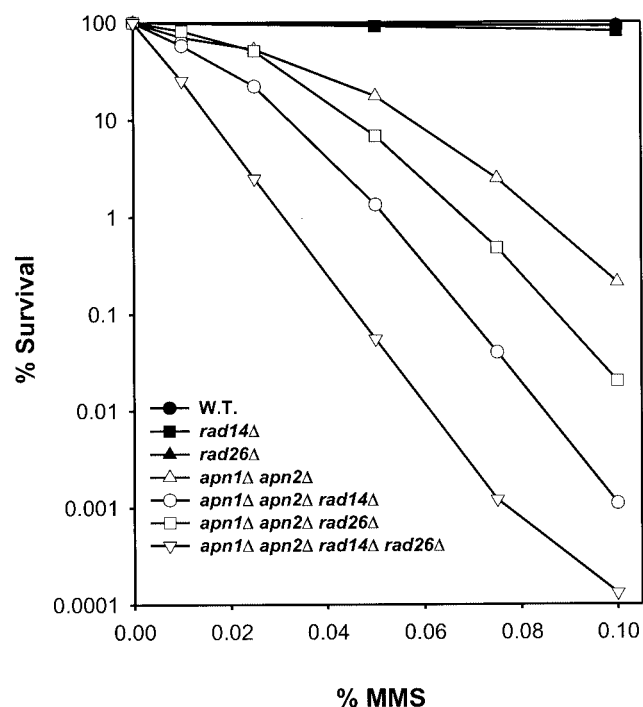
FIG. 1. Effect of the *rad26Δ* mutation on survival of MMS-treated yeast cells lacking the *APN1* and *APN2* genes or, in addition, also lacking the *RAD14* gene. Cells were treated with the indicated amounts of MMS (percentages, vol/vol) for 20 min at 30°C, following which MMS was inactivated with sodium thiosulfate and appropriate dilutions of cells were plated on YPD for viability determinations. The survival curves represent an average of three experiments for each strain. W.T., wild type.

RESULTS

Inactivation of *RAD26* enhances the MMS sensitivity of mutants defective in the removal of AP sites. *RAD26* promotes TCR of UV lesions in conjunction with the NER proteins. Hence, introduction of the *rad26Δ* mutation into any of the NER-defective mutants, such as the *rad14Δ* mutant strain, causes no further increase in the UV sensitivity of NER mutants. To determine if Rad26 functions in conjunction with Apn1, Apn2, or NER for the removal of AP sites from the transcribed strand, we treated yeast cells with the alkylating agent MMS. MMS alkylates the bases in DNA, in particular, adenine at the N3 position, forming 3-methyladenine, and guanine at the N7 position, forming 7-methylguanine. In yeast, an *N*-methylpurine DNA glycosylase encoded by the *MAG1* gene removes these and various other damaged bases (1). The resulting AP site is then removed by the Apn1 or Apn2 endonucleases or by NER (8, 20).

MMS sensitivity is greatly enhanced in the *apn1Δ apn2Δ* double mutant strain, and a further increase in MMS sensitivity occurs upon the introduction of the *rad14Δ* mutation into this strain (Fig. 1; see also reference 20). Interestingly, even though the *rad26Δ* mutation confers no perceptible increase in MMS sensitivity at the concentrations used for these experiments, introduction of the *rad26Δ* mutation into the *apn1Δ apn2Δ* or *apn1Δ apn2Δ rad14Δ* mutant strain enhances the MMS sensitivity of these strains (Fig. 1). These observations suggested

that Rad26 promotes survival of cells harboring AP sites in their DNA by a mechanism that acts independently of Apn1, Apn2, or NER.

Inhibition of transcription in MMS-treated *rad26Δ* mutant cells lacking the *APN1*, *APN2*, and *RAD14* genes for the removal of AP sites. The independence of Rad26 function from the roles of Apn1, Apn2, and NER in the removal of AP sites raised the possibility that Rad26 might enable Pol II to transcribe through AP sites. In that case, although we might expect transcription to be reduced in the *apn1Δ apn2Δ* and *apn1Δ apn2Δ rad14Δ* mutant strains because of the persistence of AP sites in DNA, transcription should be more severely curtailed in a strain lacking the various pathways for the removal of AP sites and also lacking *RAD26* because, in such a strain, Pol II will continue to stall at AP sites that would persist in DNA.

To investigate the role of *RAD26* in promoting transcription through AP sites, we examined the synthesis of galactose-inducible *GAL7* and *GAL10* mRNAs in the wild-type and *rad14Δ*, *apn1Δ apn2Δ*, *apn1Δ apn2Δ rad14Δ*, *rad26Δ*, *apn1Δ apn2Δ rad26Δ*, and *apn1Δ apn2Δ rad14Δ rad26Δ* mutant strains treated with MMS. As shown in Fig. 2, transcription of both *GAL* genes was reduced in the *apn1Δ apn2Δ* strain and a further reduction in transcription occurred in the *apn1Δ apn2Δ rad14Δ* strain. Transcription was also reduced in the *rad26Δ* strain. Transcription in the *apn1Δ apn2Δ rad26Δ* strain, however, was reduced to a greater degree than in the *apn1Δ apn2Δ* or *rad26Δ* strain, and a more severe inhibition of transcription occurred in the *apn1Δ apn2Δ rad14Δ rad26Δ* strain than in the *apn1Δ apn2Δ rad14Δ* or *rad26Δ* strain. The increase in the inhibition of transcription that occurs in the *apn1Δ apn2Δ rad14Δ* mutant strain upon the inactivation of *RAD26* suggests a role for Rad26 in promoting Pol II transcription through AP sites. If Rad26 had simply acted by displacing Pol II from AP sites and by subsequently promoting the recruitment of the repair machinery, akin to the role of *E. coli* Mfd in the TCR of thymine dimers, we would have expected transcription inhibition in the *apn1Δ apn2Δ rad14Δ rad26Δ* strain to be no greater than in the *apn1Δ apn2Δ rad14Δ* strain. That is because transcription would have continued to stall at AP sites that remain in DNA in the absence of all three pathways for their removal, regardless of whether *RAD26* is present or not.

Highly enhanced mutagenesis in MMS-treated *rad26Δ* mutant cells lacking the *APN1*, *APN2*, and *RAD14* genes. The reduction in the efficiency of transcription in the *apn1Δ apn2Δ rad26Δ* strain and the further inhibition of transcription in the *apn1Δ apn2Δ rad14Δ rad26Δ* strain implies that in these mutant strains, transcription stalls at AP sites that accumulate in DNA in the absence of the various pathways for their removal. This observation has presented the opportunity to determine if such transcriptional stalling has mutagenic consequences. To examine this, cells were treated with MMS and the frequency of MMS-induced *CAN1*^S^-to-*can1*^r^ forward mutations was determined in various mutant strains. As we have shown previously (8, 20), and show here in Fig. 3A for comparison, the frequency of MMS-induced *can1*^r^ mutations is elevated in the *apn1Δ apn2Δ* strain and a further increase in the *can1*^r^ mutation frequency occurs in the *apn1Δ apn2Δ rad14Δ* strain. Since AP sites are noncoding lesions, translesion synthesis through
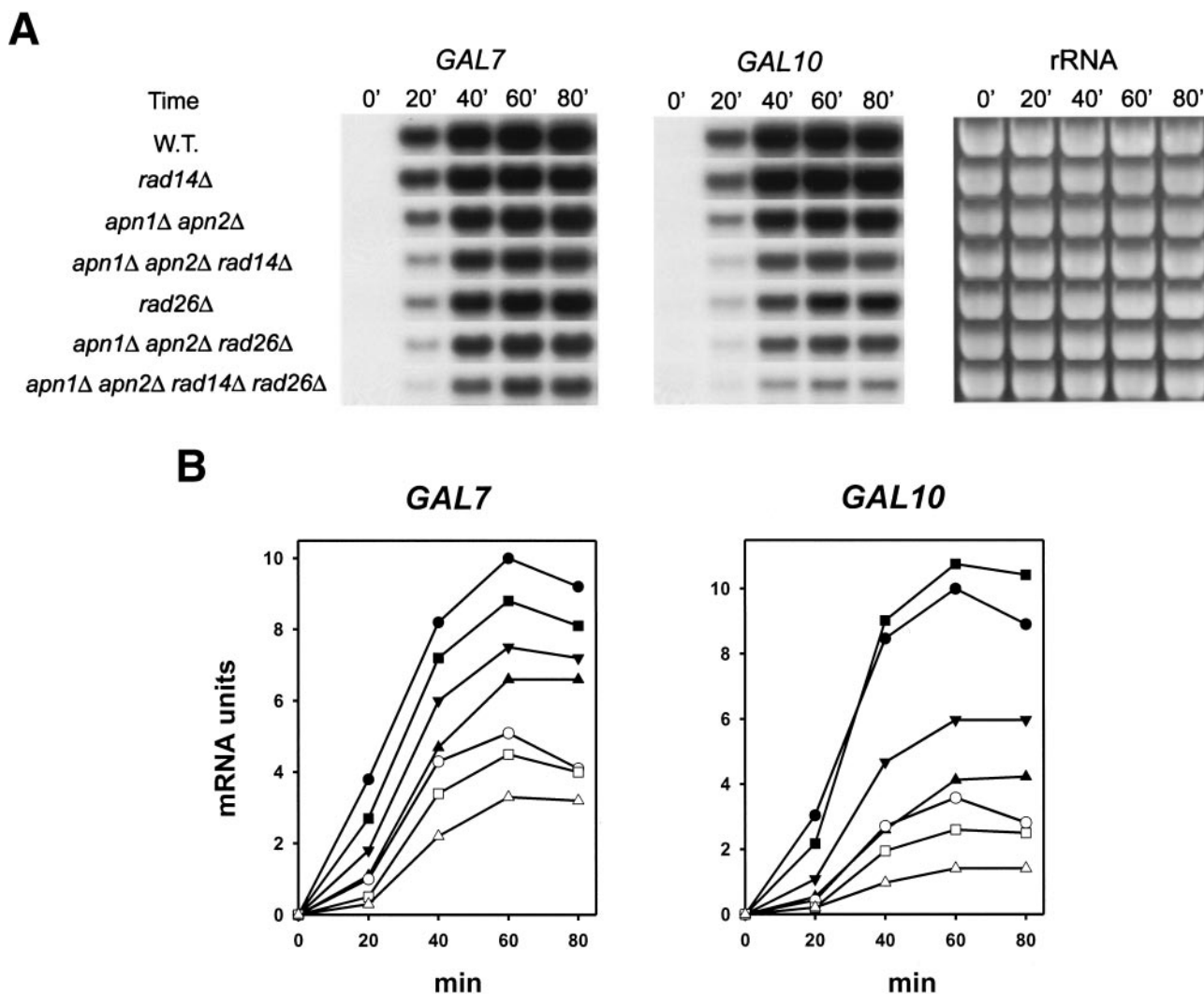
FIG. 2. Transcription of *GAL7* and *GAL10* genes in MMS-treated wild-type (W.T.) and mutant strains. Total RNAs from cells grown in YPL medium containing galactose and MMS were subjected to Northern analyses. (A) Transcript levels of *GAL7* (left) and *GAL10* (middle) genes. The ethidium bromide-stained gel (right) indicates the levels of RNAs loaded. mRNA levels were examined at the indicated times after transfer of cells to galactose- and MMS-containing medium. (B) Quantitation of *GAL7* and *GAL10* mRNA levels. mRNA units at each time point are relative to the highest mRNA level in the wild-type strain. Symbols: ●, wild type; ■, *rad14Δ* mutant; ▲, *rad26Δ* mutant; ▼, *apn1Δ apn2Δ* mutant; ○, *apn1Δ apn2Δ rad14Δ* mutant; □, *apn1Δ apn2Δ rad26Δ* mutant; △, *apn1Δ apn2Δ rad14Δ rad26Δ* mutant.

them is highly mutagenic and in yeast, *REV3*-encoded DNA polymerase ζ is indispensable for the mutagenic bypass of these lesions (8).

Even though transcription is reduced in MMS-treated *rad26Δ* cells, MMS induces no further increase in mutagenesis in the *rad26Δ* strain beyond that seen in the wild-type strain (Fig. 3A). This suggests that transcriptional stalling at MMS-induced DNA lesions, such as 3-methyladenine and 7-methylguanine, that would abound in this genetic background, is not particularly mutagenic. In striking contrast, a very large increase in the frequency of MMS-induced *can1*[r] mutations occurs in the *apn1Δ apn2Δ rad26Δ* strain over that in the *apn1Δ apn2Δ* mutant strain. For example, in comparison to the frequency of ~700 *can1*[r] mutations per 10^7 cells in the *apn1Δ apn2Δ* strain treated with 0.05% MMS, the frequency rises to over 20,000 *can1*[r] mutations per 10^7 cells in the similarly

treated *apn1Δ apn2Δ rad26Δ* strain (Fig. 3A). To ascertain that the greatly enhanced mutagenesis in the *apn1Δ apn2Δ rad26Δ* strain was, in fact, due to the *rad26Δ* mutation, we introduced the wild-type *RAD26* gene carried on a low-copy-number *CEN/ARS* plasmid into this strain and showed that the frequency of MMS-induced *can1*[r] mutations was now reduced to the level seen in the *apn1Δ apn2Δ* strain (data not shown). A large increase in the frequency of *can1*[r] mutations was also observed in the *apn1Δ apn2Δ rad14Δ rad26Δ* strain, compared to that in the *apn1Δ apn2Δ rad14Δ* strain. For example, the frequencies of *can1*[r] mutations in the *apn1Δ apn2Δ rad14Δ* mutant strain treated with 0.025 and 0.05% MMS were ~300 and 2,800/10^7 cells, respectively, whereas the corresponding frequencies in the *apn1Δ apn2Δ rad14Δ rad26Δ* strain were ~15,000 and 41,000 *can1*[r] mutants per 10^7 cells, respectively (Fig. 3A). From these observations, we infer that AP sites that remain in
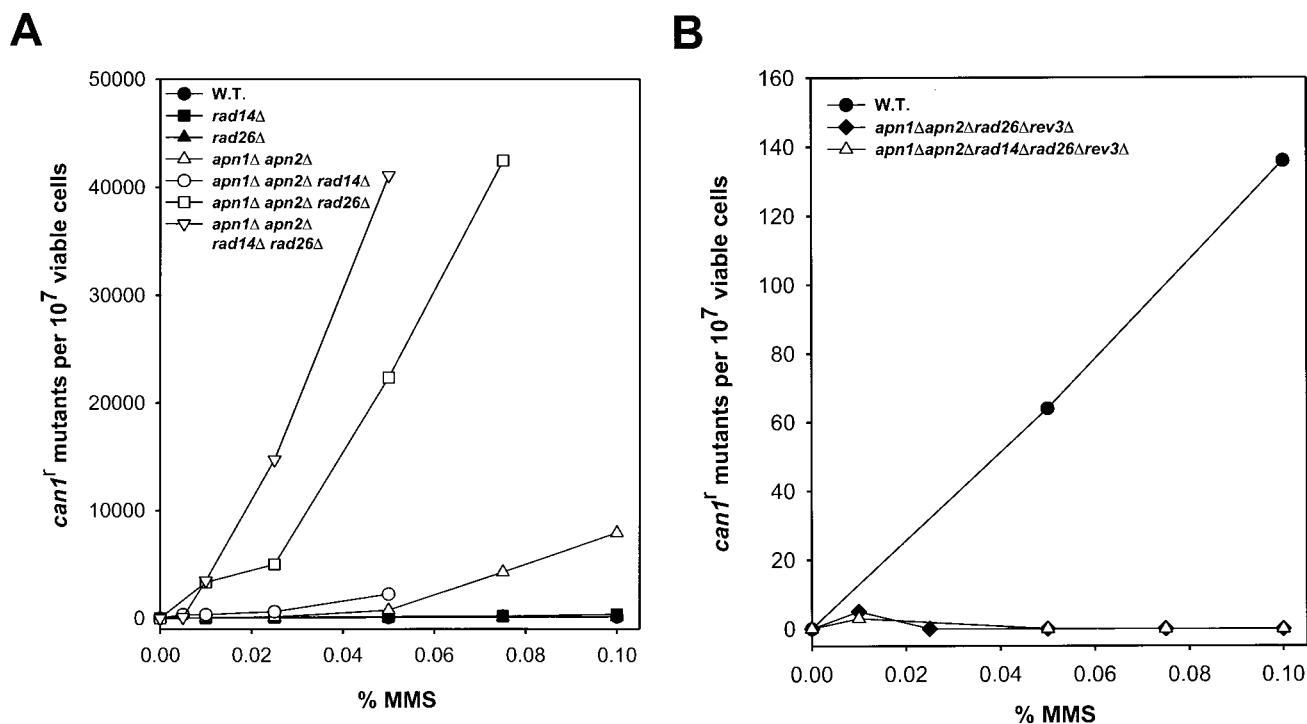
A

B

FIG. 3. MMS-induced *can1*[r] mutations in various mutant strains. Cells were treated with MMS at the indicated concentrations for 20 min at 30°C, and following inactivation of the MMS, cells were plated on YPD medium for viability determination and on synthetic complete medium lacking arginine and containing canavanine for determination of the *can1*[r] mutation frequency. Each curve represents the average of two or three experiments. (A) Elevated mutability in *rad26*Δ mutant strains lacking the *APN1*, *APN2*, and *RAD14* genes required for the removal of AP sites. (B) Requirement of *REV3* for mutagenesis in the *apn1*Δ *apn2*Δ *rad26*Δ and *apn1*Δ *apn2*Δ *rad14*Δ *rad26*Δ mutant strains. W.T., wild type.

DNA in the absence of Apn1, Apn2, and NER become highly mutagenic in cells lacking a functional *RAD26* gene.

Elevated spontaneous mutability in *rad26*Δ mutant strains lacking the pathways for AP lesion removal. AP sites arise in DNA as a result of spontaneous hydrolysis of the glycosylic bond or as an intermediate in BER. Although, in the absence of MMS treatment, we have seen no evidence of a decrease in the transcription of the *GAL7* and *GAL10* genes in the *apn1*Δ *apn2*Δ *rad26*Δ or *apn1*Δ *apn2*Δ *rad14*Δ *rad26*Δ strain compared with that in the *rad26*Δ strain (data not shown), we reasoned that, despite the infrequent occurrence of AP sites in untreated cells, it may still be possible to determine the effects of the stalling of transcription at these sites on spontaneous mutability.

Table 1 presents the rates of spontaneously occurring *can1*[r] mutations in the wild-type strain and in various mutant yeast strains. Compared to that of the wild type, mutation rates increase about twofold in the *apn1*Δ *apn2*Δ strain but show no increase in the *rad26*Δ strain. In striking contrast, *can1*[r] mutations occur in the *apn1*Δ *apn2*Δ *rad26*Δ strain at a rate that is >40-fold higher than the rate in the wild-type strain. That the large increase in the mutation rate in the *apn1*Δ *apn2*Δ *rad26*Δ strain was due to the *rad26*Δ mutation was verified by observing that the introduction of the wild-type *RAD26* gene carried on a low-copy-number yeast plasmid into the *apn1*Δ *apn2*Δ *rad26*Δ strain lowered the mutation rate very substantially. Mutation rates were also higher in the *apn1*Δ *apn2*Δ *rad14*Δ *rad26*Δ strain than in the *apn1*Δ *apn2*Δ *rad14*Δ strain.

To determine the spectrum of spontaneous *can1*[r] mutations generated in the *apn1*Δ *apn2*Δ *rad26*Δ strain, we amplified the *can1* gene from 30 independent, spontaneously arising canavanine-resistant colonies by PCR and sequenced the entire gene. The changes in the *apn1*Δ *apn2*Δ *rad26*Δ strain

TABLE 1. Rates of spontaneous *CAN1*[S] to *can1*[r] mutations in various mutant yeast strains

Strain	*can1*[r] mutation rate (10^{-7})	Fold increase
Wild type	4.4 ± 0.8	1.0
*apn1*Δ *apn2*Δ	10.2 ± 1.7	2.3
*rad14*Δ	15.0 ± 3.4	3.4
*apn1*Δ *apn2*Δ *rad14*Δ	32.8 ± 6.1	7.4
*rad26*Δ	3.3 ± 0.5	0.74
*apn1*Δ *apn2*Δ *rad26*Δ	185.5 ± 58.6	42.0
*apn1*Δ *apn2*Δ *rad26*Δ(p*RAD26*)[a]	25.4 ± 4.0	5.7
*apn1*Δ *apn2*Δ *rad14*Δ *rad26*Δ	367.6 ± 70.0	83.0
*rev3*Δ	3.3 ± 0.7	0.75
*apn1*Δ *apn2*Δ *rev3*Δ	7.5 ± 2.0	1.7
*apn1*Δ *apn2*Δ *rad14*Δ *rev3*Δ	9.0	2.0
*apn1*Δ *apn2*Δ *rad26*Δ *rev3*Δ	5.0 ± 0.7	1.1
*apn1*Δ *apn2*Δ *rad14*Δ *rad26*Δ *rev3*Δ	19.6 ± 4.9	4.4
*pol32*Δ	12.6 ± 0.6	2.9
*apn1*Δ *apn2*Δ *pol32*Δ	14.9 ± 2.8	3.4
*apn1*Δ *apn2*Δ *rad26*Δ *pol32*Δ	746.5 ± 78.8	168.5
*apn1*Δ *apn2*Δ *rad26*Δ *pol32*Δ *rev3*Δ	20.8 ± 8.6	4.7

[a] *apn1*Δ *apn2*Δ *rad26*Δ(p*RAD26*) denotes the presence of the wild-type *RAD26* gene carried on a plasmid in the *apn1*Δ *apn2*Δ *rad26*Δ mutant strain.

exhibited a prevalence of C · G-to-T · A transitions (56% of all base changes), where the original cytosine was present in the transcribed strand. The C · G-to-T · A mutations could have been formed by the incorporation of adenine opposite an AP site originating from cytosine, most likely because of its deamination to uracil, followed by the removal of uracil by a DNA glycosylase. The other base changes that we observed could be accounted for by the insertion of nucleotides other than A opposite the AP site resulting from the deamination of cytosine present in the transcribed strand or from the insertion of an A or a C opposite the AP site resulting from the spontaneous loss of G or A from the transcribed strand.

Genetic control of elevated mutagenesis in rad26Δ mutant strains lacking the pathways for AP lesion removal. Mutagenesis resulting from the replication of DNA containing AP sites is dependent upon *REV3*-encoded DNA polymerase ζ (5, 8). We found that the highly elevated mutagenesis that occurs in the *apn1Δ apn2Δ rad26Δ* and *apn1Δ apn2Δ rad14Δ rad26Δ* strains also requires polymerase ζ, since the introduction of the *rev3Δ* mutation into these strains led to a large reduction in the frequency of MMS-induced *can1*[r] mutations (Fig. 3B). The rates of spontaneous *can1*[r] mutations also declined dramatically in these mutant strains in the absence of *REV3* (Table 1).

Although AP site-induced mutagenesis resulting from replicative bypass or transcriptional stalling requires polymerase ζ, the two processes differ in their requirements for the Pol32 subunit of DNA polymerase δ. Yeast polymerase δ is composed of three subunits of 125, 58, and 55 kDa, which are encoded by the *POL3*, *POL31*, and *POL32* genes, respectively (2). By contrast to *POL3* and *POL31*, which are essential for viability, *POL32* is not essential (2); inactivation of *POL32*, however, causes a deficiency in damage-induced mutagenesis. The *pol32Δ* mutation is UV sensitive and deficient in UV mutagenesis (7), and MMS-induced mutagenesis that would result from the replication of DNA containing AP lesions is not observed in the *apn1Δ apn2Δ pol32Δ* strain (5). We have previously suggested that the requirement of the Pol32 subunit for mutagenic translesion synthesis during replication obtains from its role in connecting polymerase δ bound to the non-damaged DNA strand with polymerase ζ bound to the damaged strand (5). As shown in Table 1, the rate of spontaneous *can1*[r] mutations in the *apn1Δ apn2Δ pol32Δ* strain was about the same as in the *pol32Δ* strain, $\sim 0.14 \times 10^{-5}$; however, and in striking contrast, the rate of spontaneous *can1*[r] mutations rose to 7.5×10^{-5} in the *apn1Δ apn2Δ rad26Δ pol32Δ* strain, compared to the rate of 1.85×10^{-5} in the *apn1Δ apn2Δ rad26Δ* strain. Similarly, MMS-induced mutations also occur in the *apn1Δ apn2Δ rad26Δ pol32Δ* strain. For example, in the *apn1Δ apn2Δ pol32Δ* and *apn1Δ apn2Δ rad26Δ pol32Δ* strains treated with 0.001% MMS, the frequencies of induced *can1*[r] mutations were $\sim 9 \times 10^{-7}$ and 450×10^{-7}, respectively. Thus, by contrast to the requirement of *POL32* for mutagenic translesion synthesis through AP sites occurring during replication, this subunit is dispensable for mutagenesis resulting from the stalling of transcription at AP sites. The spontaneous and MMS-induced mutagenesis occurring in the *apn1Δ apn2Δ rad26Δ pol32Δ* strain is polymerase ζ dependent (Table 1 and data not shown).

DISCUSSION

Rad26 functions independently of BER and NER in promoting transcription through AP sites. For the repair of UV lesions, *RAD26* functions together with the components of NER, and hence, the introduction of the *rad26Δ* mutation into any of the NER-defective mutants, such as, for example, the *rad14Δ* mutant, causes no further increase in UV sensitivity. Here we show that introduction of the *rad26Δ* mutation into the *apn1Δ apn2Δ* and *apn1Δ apn2Δ rad14Δ* strains, which lack both the AP endonucleases or, in addition, also lack the NER system for the removal of AP sites, enhances the MMS sensitivity of these strains. These observations suggested that *RAD26* promotes survival of cells harboring AP sites by a mechanism that acts independently of the BER and NER processes.

Because Rad26 promotes TCR of UV-induced DNA lesions in conjunction with the NER proteins, transcription is greatly inhibited in UV-irradiated, NER-defective mutants and the inhibition of transcription that occurs in the NER-defective mutants far exceeds that seen in the *rad26Δ* strain (13). Although the mechanism of Rad26 action in TCR is not known, it presumably functions in a manner analogous to that of the Mfd protein in *E. coli*. In that case, we would expect transcription to remain inhibited in the absence of any of the essential NER proteins, as is observed. That will occur because in spite of the displacement of Pol II from UV lesions, transcription will continue to stall because of the persistence of lesions in DNA. Here we show that after MMS treatment, the levels of *GAL7* and *GAL10* transcripts are reduced in *apn1Δ apn2Δ* cells and a further reduction occurs in *apn1Δ apn2Δ rad14Δ* cells. Transcription is also reduced in *rad26Δ* cells; however, and importantly, the *apn1Δ apn2Δ rad26Δ* strain displays a much greater reduction in transcription than that seen in the *apn1Δ apn2Δ* or *rad26Δ* strain and a more severe inhibition of transcription occurs in the *apn1Δ apn2Δ rad14Δ rad26Δ* strain than in the *apn1Δ apn2Δ rad14Δ* or *rad26Δ* strain. The increase in transcriptional inhibition that occurs in the absence of *RAD26* in the *apn1Δ apn2Δ rad14Δ* strain, which lacks all of the major pathways for AP site removal, suggests that Rad26 enables Pol II to transcribe through AP sites and that, in this role, it acts independently of the Apn1, Apn2, and NER proteins.

Stalling of transcription at AP sites is highly mutagenic. The severe inhibition of transcription that we observed in the MMS-treated *apn1Δ apn2Δ rad26Δ* and *apn1Δ apn2Δ rad14Δ rad26Δ* strains, which must result from the stalling of Pol II at AP sites, has enabled us to determine the mutagenic consequences of such stalling. We show here that the frequency of MMS-induced *can1*[r] mutations is highly elevated in the *apn1Δ apn2Δ* and *apn1Δ apn2Δ rad14Δ* strains which lack the *RAD26* gene, and the rate of spontaneous *can1*[r] mutations is also much higher in the *apn1Δ apn2Δ rad26Δ* and *apn1Δ apn2Δ rad14Δ rad26Δ* strains than in the *apn1Δ apn2Δ* and *apn1Δ apn2Δ rad14Δ* strains, respectively.

Polymerase ζ is indispensable for the mutagenic bypass of AP sites during replication (5, 8). Here we show that mutagenesis in the *apn1Δ apn2Δ rad26Δ* or *apn1Δ apn2Δ rad14Δ rad26Δ* mutant strain is also dependent upon polymerase ζ. However, by contrast to the requirement of the Pol32 subunit

of polymerase δ for mutagenic translesion synthesis through AP sites during replication (5), we found that enhanced mutability in the *apn1Δ apn2Δ rad26Δ* strain does not require *POL32*. Thus, although mutagenic translesion synthesis through AP sites during both replication and transcription requires polymerase ζ, the two processes differ in their requirements for *POL32*. The requirement of *POL32* for mutagenic translesion synthesis during replication may obtain from its role in connecting polymerase δ bound to the nondamaged strand to polymerase ζ bound to the damaged strand, as we have suggested before (5).

The mutagenic bypass of AP sites during replication involves the sequential action of two DNA polymerases, in which one polymerase inserts the nucleotide opposite the lesion and polymerase ζ then extends from the inserted nucleotide (5). For the insertion reaction, polymerase δ will be a key player because of its proximity to the lesion site and also because of its proficiency at inserting an A opposite an AP site, which is the nucleotide inserted most often, as was inferred from the analyses of *can1*[r] mutations arising in MMS-treated *apn1Δ apn2Δ* cells (5). The nucleotides G, C, and T are also incorporated, but much less frequently, and polymerase η and Rev1 have been suggested to contribute to the insertion step but in a much less prominent manner than polymerase δ (5).

Similar to the *can1*[r] mutations arising from translesion synthesis through AP sites during replication, spontaneous *can1*[r] mutations in the *apn1Δ apn2Δ rad26Δ* mutant strain also occur predominantly by the incorporation of an A opposite the AP site and much less frequently by the incorporation of nucleotides other than A. This suggests that following the stalling of Pol II at an AP site, mutagenic translesion synthesis opposite this lesion also occurs by the sequential action of two different DNA polymerases, in which polymerase ζ extends from the A nucleotide inserted by polymerase δ or from the other nucleotides inserted by polymerase η or Rev1.

A model of hypermutability resulting from the stalling of transcription at AP sites. All of our observations—the much greater inhibition of transcription in the *apn1Δ apn2Δ* and *apn1Δ apn2Δ rad14Δ* strains in the absence of *RAD26* than in its presence, the much higher mutagenesis in the *apn1Δ apn2Δ rad26Δ* and *apn1Δ apn2Δ rad14Δ rad26Δ* strains than in the *apn1Δ apn2Δ* and *apn1Δ apn2Δ rad14Δ* strains, respectively, and our inference that most of the base changes occurring among spontaneous *can1*[r] mutants obtained from the *apn1Δ apn2Δ rad26Δ* strain can be accounted for by the incorporation of a nucleotide opposite AP sites present in the transcribed strand—indicate that an AP site in the transcribed strand presents a block to Pol II but that this block can be overcome by a process involving the Rad26 protein. Thus, in the absence of the pathways for the removal of AP lesions and the Rad26 protein, transcription stalls at AP sites and this stalling at AP sites is highly mutagenic.

We suggest that in a Pol II ternary complex stalled at an AP site, the nontranscribed DNA strand becomes subject to nicking by single-stranded DNA endonucleases and subsequent exonucleolytic degradation creates a gap that spans the AP site present in the transcribed strand. Thereafter, repair synthesis by the sequential action of two DNA polymerases, in which one polymerase, such as polymerase δ, polymerase η, or Rev1, inserts the nucleotide opposite the AP site and polymerase ζ

then extends from the inserted base, completes the repair process (5).

During DNA replication, the presence of a closely aligned sister duplex provides an error-free means, such as recombination (14) or a copy choice type of DNA synthesis (6), by which to bypass lesions. The very high mutagenicity of transcriptional stalling at AP sites, compared to that resulting from the replicative bypass of this lesion, may arise from the relative lack of such alternate error-free lesion bypass pathways during transcription.

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