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Mfd Is Required for Rapid Recovery of Transcription following UV-Induced DNA Damage but Not Oxidative DNA Damage in *Escherichia coli*

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Transcription-coupled repair (TCR) is a cellular process by which some forms of DNA damage are repaired more rapidly from transcribed strands of active genes than from nontranscribed strands or the overall genome. In humans, the TCR coupling factor, CSB, plays a critical role in restoring transcription following both UV-induced and oxidative DNA damage. It also contributes indirectly to the global repair of some forms of oxidative DNA damage. The *Escherichia coli* homolog, Mfd, is similarly required for TCR of UV-induced lesions. However, its contribution to the restoration of transcription and to global repair of oxidative damage has not been examined. Here, we report the first direct study of transcriptional recovery following UV-induced and oxidative DNA damage in *E. coli*. We observed that mutations in *mfd* or *uvrA* reduced the rate that transcription recovered following UV-induced damage. In contrast, no difference was detected in the rate of transcription recovery in *mfd*, *uvrA*, *fpg*, *nth*, or *polB dinB umuDC* mutants relative to wild-type cells following oxidative damage. *mfd* mutants were also fully resistant to hydrogen peroxide (H_2O_2) and removed oxidative lesions from the genome at rates comparable to wild-type cells. The results demonstrate that Mfd promotes the rapid recovery of gene expression following UV-induced damage in *E. coli*. In addition, these findings imply that Mfd may be functionally distinct from its human CSB homolog in that it does not detectably contribute to the recovery of gene expression or global repair following oxidative damage.

Xeroderma pigmentosum (XP) and Cockayne syndrome (CS) are heritable diseases with distinct clinical outcomes stemming from defects in nucleotide excision repair. Both diseases are typically associated with sunlight sensitivity. Yet, XP patients are predisposed to skin cancers, whereas CS patients have severe neurological and developmental defects (reviewed in reference 35). Classical XP results from defects in any one of 7 genes, *XPA* to *XPG*, and is characterized by an inability to remove bulky lesions from the overall genome, sometimes referred to as a defect in global genome repair (GGR) (2, 12, 30, 31, 55, 61, 71). In contrast, mutations in *CSA* and *CSB* are associated with CS and are specifically defective in removing bulky adducts from the template strand of actively transcribed genes, a process termed transcription-coupled repair (TCR) (34, 60, 64, 70). TCR also requires the participation of 5 of the 7 XP genes, *XPA*, *XPB*, *XPD*, *XPF*, and *XPG*.

While CS is sometimes considered a deficiency in a subpathway of nucleotide excision repair, the clinical outcome for individuals with this disease is, in many ways, more severe than for those with XP, which affects both pathways of nucleotide excision repair. Based on this apparent contradiction, a number of researchers have speculated that CSA and CSB may have additional functions beyond TCR of bulky lesions. Some clues to these additional roles have come from the observation that CS-B cell lines are hypersensitive to oxidizing agents, such as ionizing radiation, and are deficient in the global repair of DNA lesions induced by oxidizing agents (21, 24, 47, 65, 66). In addition, microarray analyses suggest that the absence of *CSB* leads to an impaired transcriptional response following H_2O_2 treatment (32). These observations have led researchers to propose that an underlying cause of the developmental and neurological deficiencies in CS patients may result from an abnormal accumulation of oxidative lesions, which are normally repaired by the base excision repair pathway, rather than by nucleotide excision repair (reviewed in references 13 and 27).

A series of initial reports claiming to directly demonstrate that oxidative DNA lesions in human cells are subject to TCR in a CS-dependent manner have been retracted (16, 26, 37). However, indirect evidence for TCR of oxidative lesions in mammalian cells has come from studies demonstrating that in comparison to wild-type cells, CS cell lines exhibit an increased sensitivity to H_2O_2 and fail to restore gene expression on plasmids containing the oxidative lesions 8-oxoguanine and thymine glycol (57). In addition, a role for oxidative damage in the etiology of CS has been strengthened by the recent identification of a CS-A patient exhibiting UV hypersensitivity correlating with impaired TCR of UV lesions but normal sensitivity to oxidizing agents. In this individual, mutation of W361 in CSA manifests in the mild clinical outcome of UV-sensitive syndrome without any of the severe neurological defects associated with CS (46).

TCR of bulky lesions is highly conserved and well established in *Escherichia coli* (52). Similar to humans, TCR in *E. coli* is initiated by the arrest of RNA polymerase at DNA damage and depends upon the nucleotide excision repair pathway genes (44, 54). RNA polymerase that is blocked by DNA damage serves as a signal for the coupling factor Mfd to recruit the nucleotide excision repair proteins UvrABC and initiate TCR (54). Cells lacking *mfd* or any

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of the nucleotide excision repair genes are defective in transcription-coupled repair of UV-induced damage (43, 53). However, how the absence of TCR or Mfd affects the rate that transcription recovers after UV-induced damage in *E. coli* has not been directly examined.

Whereas UV-induced lesions are strong blocks to RNA polymerases, only a subset of the lesions induced by reactive oxygen species are known to arrest prokaryotic DNA and RNA polymerases (28, 29, 49, 62, 63). In addition, base excision repair (BER) rather than nucleotide excision repair (NER) is the predominant mechanism that removes oxidative base damage, raising the possibility that BER may be recruited to repair transcriptional blocks (51). Indirect evidence for TCR at oxidative damage in *E. coli* has been inferred from studies using nongrowing cells in which inactivation of Mfd, UvrA, or Fpg glycosylase increased transcriptional mutagenesis (mutations in transcripts) opposite 8-oxoguanine lesions (8, 11). Curiously, 8-oxoguanine does not arrest RNA polymerase *in vitro* (62). Taken together, these studies would suggest a cellular role for TCR at oxidative lesions in *E. coli*. However, whether Mfd contributes to transcriptional recovery or global repair after oxidative DNA damage, similar to its CSB homolog, has not been investigated.

In this study, we measured the rate that transcription recovered in TCR-proficient and -deficient *E. coli* following UV-induced DNA damage and H_2O_2-induced oxidative damage and characterized the rate that oxidative damage was repaired in *mfd* mutants relative to wild-type cells. We found that *uvrA* and *mfd* cells were deficient in the recovery of gene expression following UV irradiation, consistent with a role for TCR in rapid restoration of transcription following UV-induced DNA damage. In contrast, the absence of Mfd did not detectably affect the transcriptional recovery following H_2O_2-induced oxidative DNA damage or contribute to the rate that oxidative lesions were removed from the genome, suggesting that Mfd may be functionally distinct from its CSB homolog in these aspects.

MATERIALS AND METHODS

Bacterial strains. The parent of all strains used in this study is SR108, a *thyA36 deoC2* derivative of W3110 (44). Isogenic strains lacking *uvrA* (HL952), *polB dinB umuDC* (CL646), *fpg* (CL1778), and *nth* (CL1006) were constructed using standard P1 transduction methods and have been described previously (18, 51). SR108 *recG* (CL559) was constructed by P1 transduction of the *recG6201*::*kan* allele from TP539 (45) into SR108. SR108 *mfd* (HL939, D. J. Crowley and P. C. Hanawalt, unpublished strain) was constructed by P1 transduction of the *mfd*::*kan* allele from WU3610-45 (53) into SR108. SR108 *mfd fpg* (CL1955) was constructed by P1 transduction of the *fpg*::*tet* allele from CL1778 into HL939. A list of the strains constructed and used in this study is shown in Table 1.

TABLE 1 *E. coli* K-12 strains used

Strain	Relevant genotype	Reference or construction
SR108	λ⁻ *thyA36 deoC2 IN*(*rrnD-rrnE*)1 *rph*	44
TP539	*recG6201*::*kan*	45
WU3610-45	*mfd-1*::*kan*	53
Strains isogenic to SR108		
HL939	*mfd-1*::*kan*	SR108 × P1 (WU3610-45), Crowley and Hanawalt, unpublished
HL952	*uvrA*::*Tn10*	18
CL559	*recG6201*::*kan*	SR108 × P1 (TP539)
CL646	*polB*::Ω Sm-Sp *dinB*::*kan umuDC595*::*cat*	17
CL1006	*nth*::*kan*	51
CL1778	*fpg*::*tet*	51
CL1955	*mfd-1*::*kan fpg*::*tet*	HL939 × P1 (CL1778)

RNA synthesis. UV irradiation used a 15-watt germicidal lamp (254 nm) at an incident dose of 0.9 J/m^2/s. For experiments using UV irradiation, overnight cultures were diluted 1:100 and grown at 37°C in Davis medium supplemented with 0.4% glucose, 0.2% Casamino Acids, and 10 μg/ml thymine (DGCthy medium) to an optical density at 600 nm (OD_{600}) of precisely 0.3, at which point one-third of the cells were mock irradiated, while the remaining culture was divided equally and irradiated at an incident dose of 50 or 100 J/m^2.

For experiments using H_2O_2 as a DNA-damaging agent, overnight cultures were diluted 1:100 and grown at 37°C in Luria Bertani medium supplemented with 10 μg/ml thymine (LBthy medium) to an OD_{600} of precisely 0.3, at which point half of the culture was mock treated, while the other half was exposed to 10 mM H_2O_2 (Fisher Scientific) for 5 min at 37°C. Following either mock or H_2O_2 treatment, catalase (Fisherbrand) was added directly to the culture to a final concentration of 200 μg/ml to remove excess H_2O_2 from the medium.

For both UV irradiation and H_2O_2 treatment, cultures were returned immediately to 37°C following exposure to allow recovery and continued growth. Duplicate 0.5-ml aliquots of culture were pulse-labeled with 0.2 μCi/ml [^{3}H]uridine for 2 min at 37°C at the times indicated. Cells were then lysed, and the DNA precipitated in cold 5% trichloroacetic acid (TCA; Fisherbrand). The precipitate was collected on Millipore glass fiber filters and the amount of ^{3}H on each filter was determined by scintillation counting.

Lesion frequency and repair rates. For UV irradiation, overnight cultures were diluted 1:100 and grown in DGCthy medium to an OD_{600} of 0.3, at which point they were UV irradiated with 50 J/m^2 and then returned to 37°C to allow recovery. For H_2O_2 challenge, overnight cultures were diluted 1:100 and grown in LBthy medium to an OD_{600} of 0.3 and treated with 10 mM H_2O_2 for 5 min at 37°C. The cells were then collected on Millipore 0.45-μm membranes, resuspended in fresh, prewarmed LBthy medium and returned to 37°C for the duration of the time course. At the times indicated, a 0.75-ml aliquot of culture was transferred to an equal volume of ice-cold NET (100 mM NaCl, 10 mM Tris [pH 8.0], 20 mM EDTA [pH 8.0]), centrifuged for 60 s, resuspended in 140-μl lysis buffer (1 mg/ml lysozyme, 0.5 mg/ml RNase A in 10 mM Tris, 1 mM EDTA [pH 8.0]), and incubated at 37°C for 30 min. Ten microliters of 10 mg/ml proteinase K and 10 μl of 20% Sarkosyl were then added to the samples, and incubation continued for a further 30 min. Samples were then extracted with four volumes of phenol-chloroform-isoamyl alcohol (25:24:1), followed by four volumes of chloroform-isoamyl alcohol (24:1) and then dialyzed against 200 ml of 1 mM Tris (pH 8.0), 2 mM EDTA (pH 8.0) for 45 min using 47-mm Millipore 0.025-μm-pore-size disks. For UV-irradiated samples, 15 μl of each DNA sample was then treated in reaction buffer (12.5 mM sodium phosphate [pH 6.8], 5 mM EDTA [pH 8.0], 50 mM NaCl, 0.5 mM dithiothreitol [DTT], 0.005% Triton X-100, 0.1 mg/ml bovine serum albumin [BSA]) supplemented with either no enzyme or 2 U T4 endonuclease V (T4 endo V; Trevigen) for 1 h at 37°C. For H_2O_2-exposed samples, 15 μl of each DNA sample was then treated in reaction buffer (30 mM EDTA [pH 8.0], 22.5 mM NaCl, 5 mM Tris [pH 8.0]) supplemented with either no enzyme or 0.53 μM Fpg glycosylase for 1 h at 37°C. Enzyme preparations were titrated using purified undamaged genomic DNA as a template. The highest enzyme concentration which did not exhibit nonspecific activity on the undamaged DNA was used. For the preparations in our lab, this corresponded to 0.53 μM Fpg glycosylase and 2 U T4 endo V.

Treated samples were then electrophoresed on a 0.5% alkaline agarose gel in 30 mM NaOH, 1 mM EDTA at 30 V for 16 h, stained and visualized

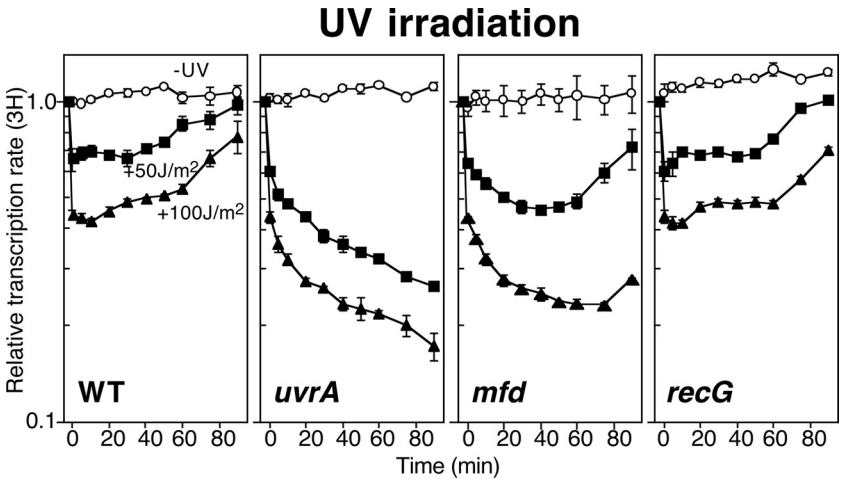

FIG 1 The transcription-coupling factor Mfd is required for rapid transcriptional recovery following UV irradiation. [^{3}H]uridine was added to cultures for 2 min at the indicated times following either 50- or 100-J/m^2 UV irradiation (filled symbols) or mock irradiation (open symbols) at time zero. The amount of RNA synthesis/2 min (^{3}H) is plotted. Graphs represent an average of at least two independent experiments. Error bars represent one standard error of the mean.

with ethidium bromide. The intensity of each high-molecular-weight band was determined using ImageQuant software (GE Healthcare). The fraction of lesion-free DNA fragments was quantified as a ratio of high-molecular-weight DNA in the T4 endo V- or Fpg-treated band to the band with no enzyme treatment at each time point. To normalize for any nicks or AP sites present in the DNA before UV or H_2O_2 exposure, the ratio at each time point was divided by the ratio at the pretreatment time point, resulting in the following formula: $(Enz_T/NoEnz_T)/R_0$. Where Enz_T is the band intensity for T4 endo V- or Fpg-treated DNA at time, *T*. $NoEnz_T$ is the band intensity for DNA with no enzyme treatment at time, *T*. R_0 is $Enz_T/NoEnz_T$ at the time immediately preceding UV irradiation or the addition of H_2O_2.

UV survival assays. Fresh overnight cultures were diluted 1:100 and grown at 37°C in DGCthy medium to an OD_{600} between 0.4 and 0.5 (approximately 6×10^8 cells/ml). Ten-μl aliquots of serial 10-fold dilutions were spotted in triplicate on LBthy plates and UV irradiated at the indicated doses. Viable colonies were counted following overnight incubation at 37°C.

H_2O_2 survival assays. Fresh overnight cultures were diluted 1:100 and grown at 37°C in LBthy medium to an OD_{600} of 0.4 (approximately 6×10^8 cells/ml) and then treated with H_2O_2 at a final concentration of 10 mM. At the times indicated, 0.1-ml aliquots of each culture were removed and serially diluted in 10-fold increments. Triplicate 10-μl aliquots of each dilution were then spotted on LBthy plates. Viable colonies were counted following overnight incubation at 37°C.

RESULTS

Mfd contributes to the rapid recovery of gene expression following UV-induced DNA damage. In mammalian cells, it has been demonstrated that a primary function of TCR is to allow cells to rapidly express genes needed to respond and recover following acute exposures to DNA damage (36, 41). The mammalian Mfd homolog, CSB, is required for the rapid recovery of transcription following UV-induced DNA damage (64). To determine whether TCR by Mfd similarly contributes to the rate that gene expression recovers after UV-induced DNA damage in *E. coli*, we monitored the rate of RNA synthesis after UV irradiation. To this end, duplicate aliquots of cultures were pulse-labeled with [^{3}H]uridine for 2 min at various times following irradiation with 50 or 100 J/m^2 of UV and the rate of transcription at each time (^{3}H incorporation/2 min) was then determined. These irradiation conditions generate approximately one cyclobutane-pyrimidine dimer (CPD) per 4.8 kb and 2.4 kb of single-strand DNA, respectively, as measured by T4 endonuclease V (T4 endo V)-sensitive sites in the DNA (19) (see Fig. 3). To ensure that any observed differences in RNA synthesis were due to UV irradiation and not culture conditions, a mock-irradiated control was included for comparison in each experiment. Under these assay conditions, the rate of synthesis in the mock-irradiated cells increased approximately 2-fold, reflecting cell growth over the course of the experiment. In contrast, the rate of RNA synthesis in UV-irradiated wild-type cells initially decreased by approximately 35% and 55% following 50- and 100-J/m^2 UV irradiation, respectively (Fig. 1). Following the initial inhibition, the recovery of transcription occurred in two distinct phases. An early phase was observed that involved a modest but reproducible increase in RNA synthesis approximately 5 min after the 50-J/m^2 dose and 20 min after the 100-J/m^2 dose. A late, more robust phase of the recovery occurred around 40 min after the 50-J/m^2 dose and 70 min after the 100-J/m^2 dose.

In UV-irradiated *uvrA* cultures, which are defective in both TCR and GGR, the rate of transcription was initially inhibited to a similar extent as in wild-type cultures; however, no subsequent recovery of RNA synthesis was observed (Fig. 1). Instead, the rate of RNA synthesis continued to gradually decline for the remainder of the 90-min time course for both of the UV doses that were examined.

In UV-irradiated *mfd* cultures, which are specifically deficient in TCR but proficient in GGR, we observed a distinct absence of the early phase of the transcription recovery compared to wild-type cells (Fig. 1). After the initial inhibition of transcription, the rate of RNA synthesis continued to decrease gradually in *mfd* mutants, similar to what was observed in UV-irradiated *uvrA* mutants. The gradual decrease continued until 75 min and 90 min following 50- and 100-J/m^2 treatments, respectively, at which time, the second phase of transcriptional recovery was observed.

As an additional control, we also examined the rate at which gene expression recovered in *recG* mutants. Similar to *mfd*, *recG* encodes a DNA helicase that shares a homologous ATP-dependent translocation domain and renders cells hypersensitive to UV-

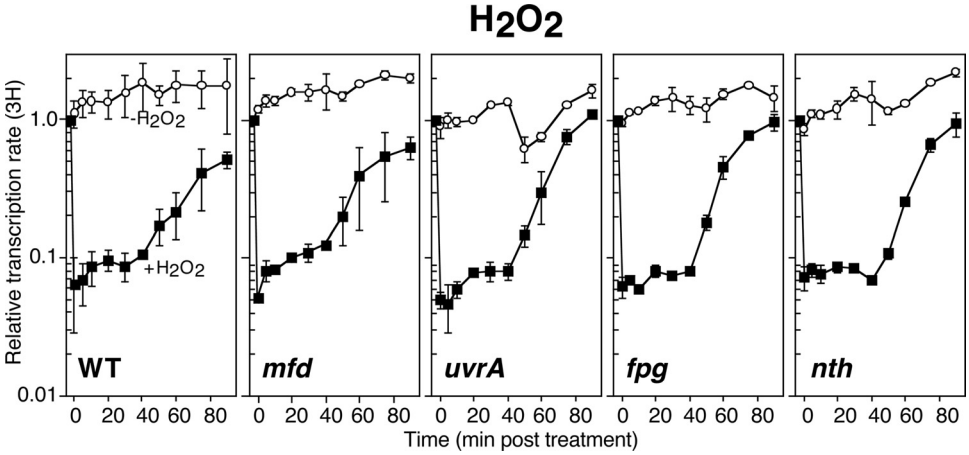

FIG 2 The absence of Mfd does not affect transcriptional recovery following H_2O_2-induced DNA damage. Cells were either exposed to 10 mM H_2O_2 for 5 min (filled symbols) or mock treated (open symbols) at time zero and then allowed to recover in the presence of 200 μg/ml catalase. At the indicated times, [^{3}H]uridine was added to cultures for 2 min. The amount of RNA synthesis/2 min (^{3}H) is plotted. Graphs represent an average of at least two independent experiments. Error bars represent one standard error of the mean.

irradiation (39). However, unlike *mfd*, *recG* is not required for TCR (1, 4, 9, 23, 59). Following UV irradiation, we observed that *recG* cells recovered gene expression with wild-type kinetics and in a similar biphasic manner (Fig. 1). The normal transcriptional recovery observed in *recG* mutants despite the elevated levels of cell lethality argues that the defect in recovering transcription in *mfd* mutants is specific to the absence of TCR and not due to the elevated levels of lethality that occur in these UV-irradiated populations.

The time at which the early and late phases of transcriptional recovery is observed correlates with the approximate period in which repair by TCR and GGR occurs, respectively (20, 44). Consistent with this, *mfd* mutants are specifically deficient in the early but not the late phase of transcriptional recovery, whereas *uvrA* mutants are defective in both phases of the recovery. These results demonstrate that Mfd in *E. coli* contributes functionally to the rapid recovery of transcription following UV-induced damage, similar to its mammalian CSB homolog.

The absence of Mfd does not delay the recovery of transcription following H_2O_2-induced DNA damage. Mammalian cell lines lacking CSB fail to restore gene expression from plasmids containing 8-oxoguanine and thymine glycol lesions (33, 57). To determine whether Mfd detectably contributes to the recovery of gene expression following oxidative damage, we examined the rate of transcriptional recovery in cultures following treatment with H_2O_2 using the assay described above. To this end, cultures were treated with 10 mM H_2O_2 for 5 min, before catalase was added to remove excess H_2O_2 from the medium and allow recovery to occur. Then, duplicate aliquots of culture were pulse-labeled with [^{3}H]uridine for 2 min at the indicated time points as before. To control for the effect of catalase and culture conditions on transcription, mock-treated cultures were included in each experiment.

By this assay, the rate of transcription increased by approximately 2-fold in mock-treated cultures of all strains over the time course of the experiment. In wild-type cultures treated with H_2O_2, we observed a pattern of transcriptional inhibition and a biphasic recovery that was similar to that seen in UV-irradiated cultures (Fig. 2). The rate of transcription initially decreased by ~90% after H_2O_2 treatment. An initial, modest recovery in the rate of transcription occurred during the first 5 min, followed by a more rapid and robust recovery that began approximately 40 min after the H_2O_2 was removed.

When we examined H_2O_2-treated *mfd* cultures, we observed that the recovery of transcription was indistinguishable from that seen in wild-type cells (Fig. 2). Fpg and endonuclease III, encoded by *fpg* and *nth*, are two of the predominant glycosylases that remove oxidative base adducts in *E. coli* (7, 22, 51). Following H_2O_2 treatment of *uvrA*, *fpg*, or *nth* mutants, transcription also recovered at rates that were similar to wild-type cells. The results demonstrate that the absence of Mfd and TCR does not detectably impair the time or rate that transcription recovers following oxidative DNA damage in *E. coli*.

Mfd does not detectably contribute to the global repair of oxidative damage following H_2O_2 treatment. Studies in mammalian cells have shown that the absence of CSB results in a deficiency in GGR of oxidatively induced lesions but not UV-induced lesions (21, 24, 47, 65–67). To determine whether Mfd affects the rate that oxidative lesions are repaired from the global genome, we monitored the removal of Fpg-sensitive sites from the DNA of H_2O_2-treated cultures over time. For comparison, the repair of UV-induced damage, as monitored by the removal of T4 endo V-sensitive sites, was also examined. To this end, cultures were either irradiated with 50 J/m^2 of UV or treated with 10 mM H_2O_2 for 5 min before cells were filtered, resuspended in fresh, prewarmed medium, and allowed to recover at 37°C. High-molecular-weight genomic DNA was purified from both cultures at the indicated time points. The DNA from each time point was then incubated with T4 endo V or Fpg glycosylase and then electrophoresed in a denaturing alkali gel. The presence of T4 endo V- or Fpg-sensitive sites in the DNA is indicated by the loss of high-molecular-weight DNA.

In all UV-irradiated cultures examined in the absence of T4 endo V treatment, purified genomic DNA remained high molecular weight at all times throughout the recovery period (Fig. 3A). DNA fragments averaged greater than 40 kb, which was the approximate limit of resolution of our agarose gels. In wild-type cultures, incubation of the DNA from preirradiated cultures with

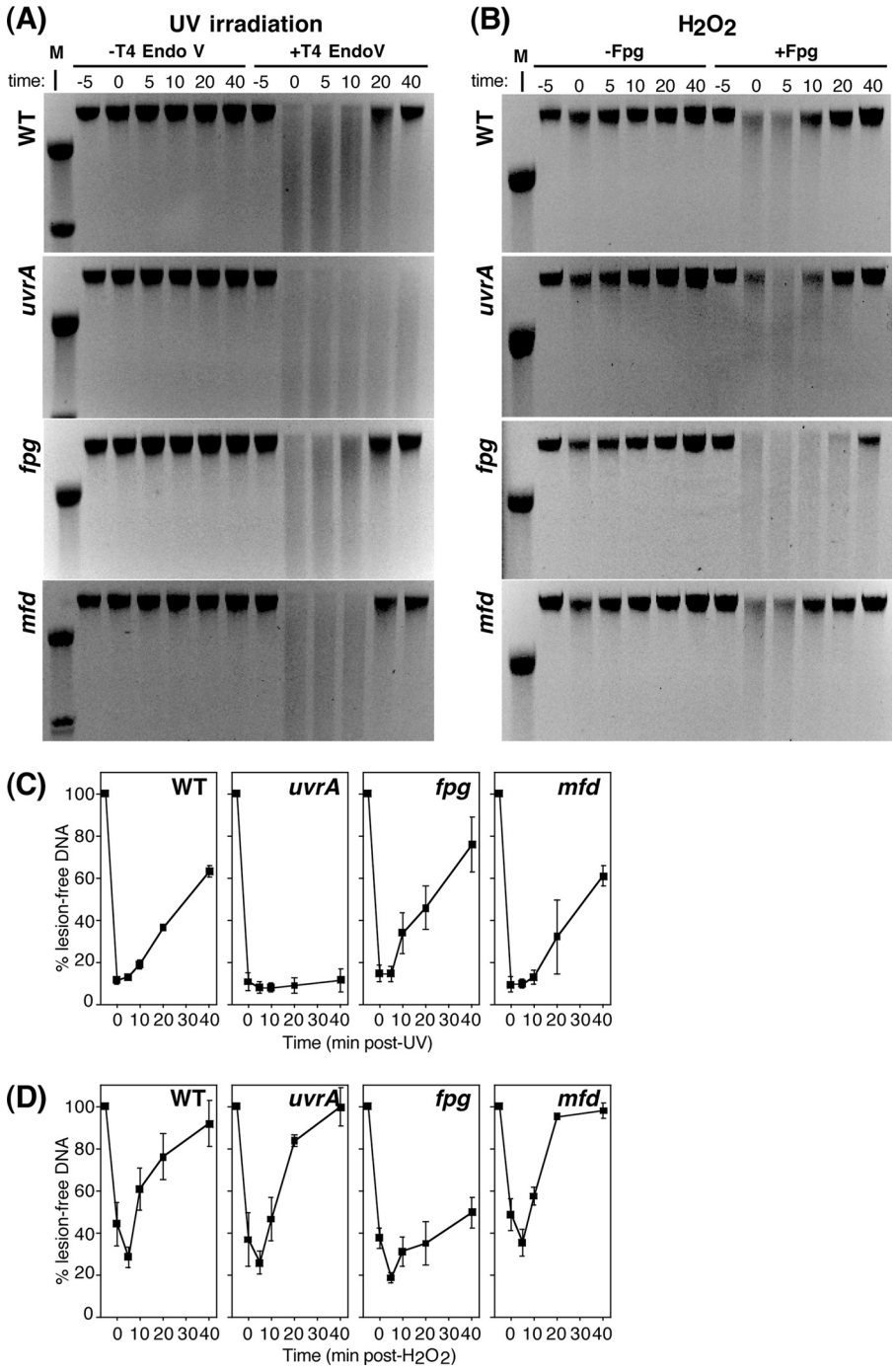

FIG 3 Mfd does not affect the global rate of repair following either UV irradiation or H_2O_2 treatment. (A) wild-type (WT; first panel), *uvrA* (second panel), *fpg* (third panel), and *mfd* (last panel) cells were UV irradiated at 50 J/m^2 and allowed to recover. At the indicated times, genomic DNA was purified and either treated with T4 endonuclease V (T4 endo V) or no T4 endo V for 1 h at 37°C and then analyzed on alkali-agarose gels. A representative gel is shown for each analyzed strain. (B) WT (first panel), *uvrA* (second panel), *fpg* (third panel), and *mfd* (last panel) cells were exposed to 10 mM H_2O_2 for 5 min and allowed to recover. At the indicated times, genomic DNA was purified and either treated with Fpg or no glycosylase for 1 h at 37°C and then analyzed on alkali-agarose gels. A representative gel is shown for each analyzed strain. (C) The percentage of lesion-free, high-molecular-weight DNA in T4 endo V-treated samples is plotted for each time point relative to mock-treated samples. (D) The percentage of lesion-free, high-molecular-weight DNA in Fpg-treated samples is plotted for each time point relative to mock-treated samples. Graphs represent an average of at least three independent experiments. Error bars represent one standard error of the mean. M, lambda HindIII molecular-weight marker, top two bands represent 23.1 and 9.4 kb, respectively.

T4 endo V resulted in little or no loss of high-molecular-weight DNA. At times immediately following UV irradiation in wild-type cells, a loss of high-molecular-weight DNA was observed in samples incubated with T4 endo V, indicating the presence of UV-induced lesions. Over time, the number of T4 endo V-recognized sites began to decrease, as lesion-free, high-molecular-weight DNA returned and by 40 min, more than 60% of the DNA had been restored (Fig. 3C). In contrast, in UV-irradiated *uvrA* cultures, which are defective in nucleotide excision repair, lesions persisted throughout the 40-min time course (Fig. 3A and C). As expected, the absence of Fpg, which is not involved in the repair of UV-induced damage, did not affect the rate that UV-induced lesions were removed from the genome. When we measured the rate of global repair in *mfd* mutants after UV irradiation, we observed a repair profile closely following the repair profile of wild-type cells (Fig. 3C).

To assess repair in wild-type, *fpg*, *uvrA*, and *mfd* cells following oxidative damage, total genomic DNA was prepared at the indicated time points after H_2O_2 exposure, and the number of Fpg-sensitive sites remaining in the DNA was determined. In samples not incubated with Fpg, genomic DNA remained high molecular weight at all time points examined, both before and after H_2O_2 treatment for all strains examined (Fig. 3B).

In wild-type cultures, DNA purified from cultures prior to H_2O_2 exposure remained high molecular weight following incubation with Fpg, demonstrating that the enzyme is specific for oxidative lesions. At times immediately following H_2O_2 treatment, there was a loss of high-molecular-weight DNA, indicating that lesions recognized by Fpg were present in the DNA (Fig. 3B). The number of Fpg-sensitive sites increased modestly within the first 5 min of the recovery period, which is likely to be due to reactive oxygen species that continue to be generated in the cell after the H_2O_2 has been removed (10, 40). Based on the average DNA fragment size in glycosylase-treated samples, the lesion frequency never exceeded more than one Fpg substrate per ~40-kb strand of DNA at any given time. However, the continued induction of DNA lesions during the repair period, the high rate of glycosylase repair, and the observed 90% inhibition of transcription suggest that the total number of lesions generated by this treatment was significantly higher than that induced after UV treatment. Despite the higher number of lesions, repair occurred rapidly. The number of Fpg-sensitive sites in H_2O_2-treated wild-type cells began to decrease by 10 min, and by 40 min, greater than 90% of the fragments were resistant to cleavage by Fpg and migrated as a high-molecular-weight band (Fig. 3D).

In cells lacking UvrA, which is not involved in the repair of oxidative DNA damage (51), Fpg-sensitive sites were removed from the genome with kinetics that were similar to wild-type cultures (Fig. 3B and D). In contrast, in *fpg* cultures exposed to H_2O_2, the lesions were seen to persist, and at 40 min, less than 50% of the DNA was resistant to Fpg treatment, consistent with Fpg's role in GGR of oxidative lesions (51). To determine if Mfd plays a role in oxidative lesion removal from the chromosome, we next examined the repair rate of Fpg-sensitive sites in *mfd* cells. After treatment with H_2O_2, *mfd* strains removed oxidative damage at a rate that was not detectably different from either wild-type or *uvrA* cultures (Fig. 3B and D).

In mammals, the CSB defect in GGR of oxidative damage renders cells hypersensitive to H_2O_2 (24, 47, 65, 66). To determine the effect of the *mfd* mutation on H_2O_2 sensitivity, the relative survival of each strain was also examined (Fig. 4). Consistent with previous reports, the absence of *mfd* rendered cells modestly hypersensitive to UV-induced damage, although to a lesser extent than that of a *recG* mutant. In contrast, *mfd* mutants appeared as resistant as wild-type cultures when exposed to H_2O_2 (Fig. 4B). Similarly, neither *fpg* nor *uvrA* mutants were hypersensitive to H_2O_2, in agreement with previous reports (3, 50, 51). Taken together, the results indicate that the absence of *mfd* does not detectably impair the global repair of oxidative damage, in contrast to what is seen with CSB in mammalian cells.

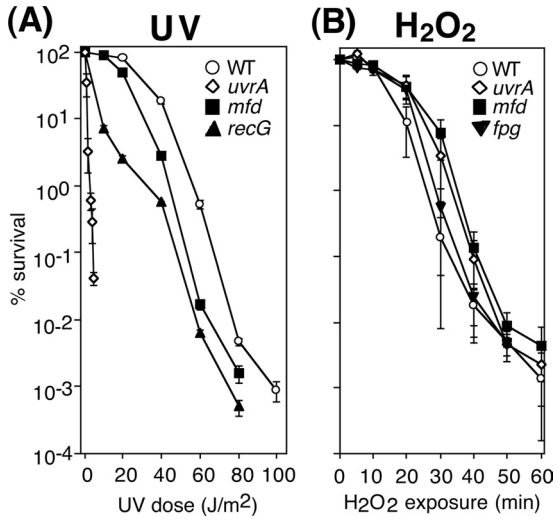

FIG 4 Mfd is hypersensitive to UV irradiation but not H_2O_2 treatment. (A) The survival rates of wild-type (○), *mfd* (■), *recG* (▲), and *uvrA* (◇) cultures are shown after UV irradiation at the indicated doses. (B) The survival rates of wild-type (○), *mfd* (■), *fpg* (▼), and *uvrA* (◇) cultures are shown after exposure to 10 mM H_2O_2 for the indicated amount of time. All graphs represent an average of at least three independent experiments. Error bars represent one standard error of the mean.

Base excision repair does not contribute to the restoration of transcription in *mfd* mutants nor is translesion DNA synthesis involved in transcriptional recovery following H_2O_2-induced DNA damage. The lack of a phenotype for *mfd* mutants in the recovery of gene expression after oxidative challenge could reflect rapid turnover of oxidative lesions by base excision repair enzymes. In such a scenario, Fpg would efficiently and rapidly remove oxidative lesions before they arrested the transcription machinery, thus masking any effect of Mfd, and by extension TCR, on the recovery of RNA synthesis after oxidative DNA damage. Analogous to this, the rapid repair of 6-4 photoproducts by GGR has been shown to obscure TCR of this lesion in mammalian cells that express wild-type XPC (69). Also consistent with the idea that efficient GGR may be masking oxidative-TCR events is the observation that transcriptional mutagenesis at 8-oxoguanine lesions is significantly higher in *fpg* mutants (~43-fold) than in *mfd* mutants (~12-fold) and synergistically increases when both *fpg* and *mfd* are inactivated (8). To examine this possibility directly, we constructed an *mfd fpg* double mutant and determined the rate of transcriptional recovery in these cultures following treatment with H_2O_2 using the assay described above. We observed that the recovery of transcription in H_2O_2-treated *mfd fpg* cultures was indistinguishable from that seen in either single mutant or wild-type cells (Fig. 5A). This result indicates that the wild-type tran-

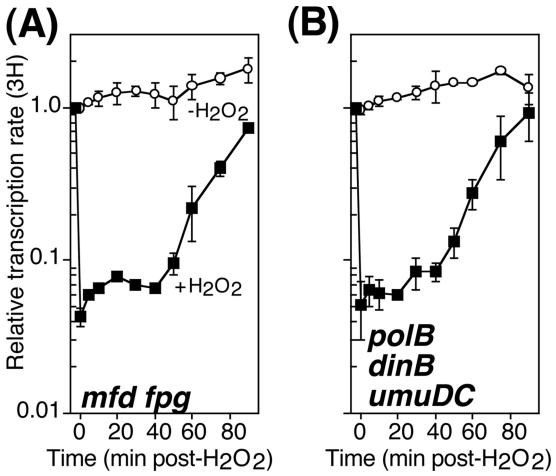

FIG 5 The lack of a requirement for *mfd* in restoring transcription after H_2O_2-induced DNA damage is not due to the high rate of base excision repair or translesion synthesis. Cells were either exposed to 10 mM H_2O_2 for 5 min (filled symbols) or mock treated (open symbols) at time zero and then allowed to recover in the presence of 200 μg/ml catalase. At the indicated times, [^{3}H] uridine was added to cultures for 2 min. The amount of RNA synthesis/2 min (^{3}H) is plotted for *mfd fpg* (A) and *polB dinB umuDC* (B) mutants. Graphs represent an average of at least two independent experiments. Error bars represent one standard error of the mean.

scriptional recovery observed in *mfd* cells is not due to efficient removal of oxidative lesions by base excision repair enzymes.

Our results suggested that the restoration of gene expression following oxidative challenge was not dependent on either *mfd*-mediated TCR or rapid *fpg*-mediated GGR. We therefore considered whether translesion DNA synthesis could be facilitating bypass of oxidative lesions on the transcribed strand. Transcription-coupled translesion synthesis has been suggested by physical and genetic interactions between the *E. coli* RNA polymerase modulator NusA and the translesion DNA polymerases Pol IV (*dinB*) and Pol V (*umuDC*) (14). This process has also been implicated in cell survival following nitrofurazone treatment in stationary cells (15). To test this possibility, we monitored the rate of RNA synthesis after H_2O_2 treatment in *polB dinB umuDC* cells, which lack all three translesion DNA polymerases. Similar to what we observed in wild-type cells, we found that gene expression was restored in a biphasic manner following oxidative challenge in *polB dinB umuDC* cells (Fig. 5B). This result indicates that translesion DNA polymerases are not contributing to the recovery of transcription following exposure to H_2O_2.

DISCUSSION

Mfd effect on UV-inactivated RNA synthesis. It has been proposed that a primary function of transcription-coupled repair is to promote the rapid recovery of gene expression following DNA damage. In this sense, TCR is proposed to act as a triage mechanism by first restoring the integrity of genes needed for the cellular response to the stress. Several studies have shown that CSA and CSB function is required to rapidly restore transcription in eukaryotic cells (36, 41, 58, 64, 68). However, the effect that TCR has on the rate of transcriptional recovery had not been directly examined in *E. coli*. The results presented here demonstrate that TCR is essential for the early recovery of RNA synthesis following UV irradiation and supports the idea that Mfd functions similarly to its CSB homolog in this respect.

The level of transcriptional inhibition following UV irradiation in *E. coli* was dose dependent and consistent with the idea that transcription is arrested following a direct collision or encounter with the UV-induced photoproduct. Immediately following UV irradiation at 50 J/m^2 (1 lesion/4.8 kb single-stranded DNA [ssDNA]) and 100 J/m^2 (1 lesion/2.4 kb ssDNA), RNA synthesis was inhibited by 35% and 55%, respectively. Assuming an average transcript length of ~1,200 bp in *E. coli*, this level is within a reasonable approximation of the 25% and 50% inhibition one would be expected to observe.

A previous study by Li and Bockrath (38) examined how the absence of Mfd affected the production of β-galactosidase, encoded by *lacZ*, and galactoside acetyl-transferase, encoded by *lacA*, after UV (38). The authors demonstrated that the absence of Mfd impaired the inducibility of these proteins after UV. At the level of protein expression, the authors found that both Mfd and nucleotide excision repair mutants were impaired to a similar extent, leading the authors to conclude that nucleotide excision repair appears to make no contribution beyond that associated with TCR in restoring gene expression. Our results complement and extend these observations in two aspects. First, using an assay to monitor the rate of transcription directly, we were able to observe distinct Mfd-dependent and -independent phases of recovery following UV-induced damage. The early phase of transcriptional recovery involved TCR and required both Mfd and nucleotide excision repair, whereas the late phase only depended on nucleotide excision repair. Second, the comparative levels of transcription inhibition and hypersensitivity between *mfd*, *uvrA*, and *recG* mutants establishes that the transcriptional defect in Mfd mutants is specific to its role in TCR and not due to an indirect product of the elevated levels of lethality that occur in these cells.

TCR effect on H_2O_2-inactivated RNA synthesis. In contrast to UV damage, the absence of *mfd* did not have a detectable effect on either the transcriptional recovery, the removal of lesions, or cell survival following oxidative DNA damage and differs from the phenotypes observed for CSB in mammalian cells. H_2O_2 is known to induce several different classes of oxidative lesions on DNA, including 8-oxoguanine, thymine glycol, and 4,6-diamino-5-formamidopyrimidine (6). Of these, a subset such as thymine glycol has been shown to arrest prokaryotic DNA and RNA polymerases *in vitro*, while others such as 8-oxoguanine do not or only partially arrest the RNA polymerase (28, 29, 49, 56, 62, 63). Given the generally accepted model that an RNA polymerase stalling event is required for the activation of the TCR pathway (42), one possible reason we were unable to detect any effect of the *mfd* mutation following H_2O_2 treatment is that nonblocking oxidative lesions predominate. This type of model would predict that Mfd may still be required to repair the lesions in transcribed genes but would not prohibit transcription from occurring in its absence.

Another possibility is that these observed differences represent a true functional divergence between Mfd and CSB. Consistent with this, a number of studies have suggested CSB has secondary functions in chromatin remodeling and basal transcription that are independent from its role in processing arrested transcription complexes (5, 25, 48). How these potential functions relate to the repair of oxidative damage or whether Mfd has analogous functions in bacteria has not yet been examined. It is also worth noting that the processing of oxidative lesions is separable from TCR in

eukaryotic cells as indicated in *CSA* W361C mutants, which have normal resistance to oxidizing agents but are defective in TCR and hypersensitive to UV lesions (46). These phenotypes closely mimic those reported here for the *mfd* mutant in *E. coli*.

A third possibility, which is not mutually exclusive, is that the absence of an effect by Mfd could reflect a growth phase-dependent activity for this protein following oxidative challenge. Consistent with this hypothesis is the indirect evidence that TCR does occur at 8-oxoguanine lesions in nongrowing *E. coli* (8). Using a novel assay to monitor mutations arising in transcripts of nonreplicating cells, Brégeon et al. found that the rate of transcriptional mutagenesis on DNA templates bearing 8-oxoguanine was elevated ~12-fold in *mfd* mutants compared to wild-type cells. A more recent study by this group and using the same mutagenesis assay with nongrowing *mfd uvrA* and *fpg uvrA* found slightly elevated transcriptional mutagenesis rates at 8-oxoguanine lesions in these double mutants compared to their respective single mutant parents, suggesting that Mfd-mediated TCR might proceed through either base excision repair or nucleotide excision repair at 8-oxoguanine (11). The finding that nucleotide excision repair enzymes can function at 8-oxoguanine lesions in nongrowing cultures (11) contrasts with what we have found in our present and previous studies in actively growing *E. coli* (51), where we observe that UvrA mutants are able to remove oxidative lesions with wild-type kinetics, and further suggests an effect of growth phase on the repair of oxidative DNA damage. In a separate study, modulation of transcription by NusA was shown to alter cell viability in the presence of nitrofurazone-induced DNA damage under nongrowth conditions (15), an observation that would also be consistent with the presence of a DNA damage repair or tolerance mechanism at the level of transcription in stationary-phase *E. coli* cells. Finally, given the predominant neurological symptoms associated with CS patients (13, 27, 57), it seems possible that the effects of TCR at oxidative lesions may also be of consequence in nondividing human cells.

A somewhat unexpected observation in our previous study is that survival and recovery of replication and transcription are only minimally affected by the absence of Fpg, even though oxidative lesions persist in the genomes of these mutants (51). Equally surprising are the results presented here that show the absence of Mfd does not affect the survival or recovery of transcription in growing cells, even when global genomic repair of the prominent oxidative lesions is disrupted. The mechanism that allows replication and transcription to recover and continue in cells containing oxidative lesions remains an interesting question for further study.

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