JOURNAL OF BACTERIOLOGY, Aug. 2009, p. 4959–4965 Vol. 191, No. 15
0021-9193/09/$08.00+0 doi:10.1128/JB.00495-09

Nucleotide Excision Repair Is a Predominant Mechanism for Processing Nitrofurazone-Induced DNA Damage in *Escherichia coli*[▽]

Katherine R. Ona,* Charmain T. Courcelle, and Justin Courcelle

Department of Biology, Portland State University, Portland, Oregon 97207

Received 9 April 2009/Accepted 18 May 2009

Nitrofurazone is reduced by cellular nitroreductases to form N^2-deoxyguanine (N^2-dG) adducts that are associated with mutagenesis and lethality. Much attention recently has been given to the role that the highly conserved polymerase IV (Pol IV) family of polymerases plays in tolerating adducts induced by nitrofurazone and other N^2-dG-generating agents, yet little is known about how nitrofurazone-induced DNA damage is processed by the cell. In this study, we characterized the genetic repair pathways that contribute to survival and mutagenesis in *Escherichia coli* cultures grown in the presence of nitrofurazone. We find that nucleotide excision repair is a primary mechanism for processing damage induced by nitrofurazone. The contribution of translesion synthesis to survival was minor compared to that of nucleotide excision repair and depended upon Pol IV. In addition, survival also depended on both the RecF and RecBCD pathways. We also found that nitrofurazone acts as a direct inhibitor of DNA replication at higher concentrations. We show that the direct inhibition of replication by nitrofurazone occurs independently of DNA damage and is reversible once the nitrofurazone is removed. Previous studies that reported nucleotide excision repair mutants that were fully resistant to nitrofurazone used high concentrations of the drug (200 μM) and short exposure times. We demonstrate here that these conditions inhibit replication but are insufficient in duration to induce significant levels of DNA damage.

Replication in the presence of DNA damage is thought to produce most of the mutagenesis, genomic rearrangements, and lethality that occur in all cells. UV-induced photoproducts, X-ray-induced strand breaks, psoralen- or *cis*-platin-interstrand cross-links, oxidized bases from reactive oxygen species, and base depurination are just a few of the structurally distinct challenges that the replication machinery must overcome. It seems likely that the mechanisms that process these lesions will vary depending on the nature of the impediment.

While a number of the lesions described above are known to block replication, the events associated with UV-induced damage have been the most extensively characterized. UV irradiation causes the formation of cyclobutane pyrimidine dimers and 6-4 photoproducts in DNA that block the progression of the replication fork (16, 29, 30, 37). Following the arrest of replication at UV-induced damage, RecA and several RecF pathway proteins are required to process the replication fork such that the blocking lesion is removed or bypassed (2, 5, 6, 8–10). Cells lacking either RecA or any of several RecF pathway proteins are hypersensitive to UV-induced damage and fail to recover replication following disruption by the lesions (2, 6, 10). RecBCD is an exonuclease/helicase complex that is involved in repairing double-strand breaks (38). It also is required for resistance to UV-induced damage, although it is not required to process or restore disrupted replication forks, and the substrates it acts upon after UV irradiation currently remain unclear (3, 10, 19).

Survival and the ability to resume DNA synthesis following UV-induced damage depend predominantly on the removal of the lesions by nucleotide excision repair (5, 7, 36). Cells deficient in nucleotide excision repair are unable to remove UV-induced DNA lesions and exhibit elevated levels of mutagenesis, strand exchanges, rearrangements, and cell lethality (16, 33, 34). In cases where replication fork processing or lesion repair is prevented, the recovery of replication and survival become entirely dependent on translesion synthesis by DNA polymerase V (Pol V) (6). However, in repair-proficient cells, the contribution of translesion synthesis to recovery and survival is minor and is detected only following UV doses that exceed the repair capacity of the cell (5, 6).

Less is known about how replication recovers from other forms of DNA damage. We chose to characterize nitrofurazone, because a number of studies suggested that N^2-deoxyguanine (N^2-dG) adducts induced by this and other agents would be processed differently than UV-induced lesions. Nitrofurazone is a topical antibacterial agent that historically has been used for treating burns and skin grafts in patients and animals (14, 15, 32). Nitrofurazone toxicity is known to require activation by cellular nitroreductases (25, 42). However, the mechanism and targets of its antimicrobial properties have yet to be fully elucidated. In addition to its antimicrobial properties, the reduced nitrofurazone metabolites also target DNA and have been shown to induce free radical damage, strand breaks, and N^2-dG adducts (26, 40, 42, 45), and they are mutagenic and carcinogenic in rodent models (1, 15, 24, 39).

Whereas nucleotide excision repair is the predominant mechanism required for survival after UV-induced damage, a number of studies suggest that translesion synthesis plays a larger role in survival after nitrofurazone-induced DNA dam-

* Corresponding author. Mailing address: Department of Biology, Portland State University, P.O. Box 751, Portland, OR 97201. Phone and fax: (503) 725-3865. E-mail: kro@pdx.edu.

▽ Published ahead of print on 22 May 2009.

age. *dinB* mutants lacking Pol IV were shown to be hypersensitive to nitrofurazone compared to cells that constitutively express the polymerase (17). Biochemically, Pol IV and a number of Pol IV homologs from other organisms have been shown to efficiently replicate over a range of N^2-dG adducts in vitro (17, 35, 44). In addition, several studies have reported that *uvrA* mutants, which are defective in nucleotide excision repair, do not exhibit any hypersensitivity to nitrofurazone or other agents that induce similar adducts in vivo (12, 21, 27). Early studies also observed a direct correlation between nitrofurazone-induced mutations and lethality, suggesting that mutagenic lesions persist in the DNA to cause toxicity (21, 23, 27, 43). Consistent with these observations, nitrofuran-induced lesions were found to be poor substrates for nucleotide excision repair in vitro (46).

Taken together, these observations suggest to us that the cellular response to nitrofurazone will be distinct from its response to UV irradiation. However, no study has examined the relative contributions that nucleotide excision repair, translesion synthesis, or recombination has in recovering from nitrofurazone-induced damage. In this study, we characterized the mechanism by which nitrofurazone inhibits DNA replication and identified the genes that contribute to the recovery, survival, and mutagenesis of *Escherichia coli* treated with nitrofurazone. In contrast to previous studies, we found that survival following nitrofurazone-induced damage depends predominantly on nucleotide excision repair. Similarly to UV-induced DNA damage, both the RecF and RecBC pathways contribute to survival following nitrofurazone-induced DNA damage. The contribution of translesion polymerases to survival was minor and was mediated by Pol IV. In addition, we found that nitrofurazone can act to inhibit DNA replication directly when used at higher concentrations. The direct inhibition of replication is reversible and occurs independently of DNA damage, suggesting that DNA is not the primary target of its antimicrobial properties.

MATERIALS AND METHODS

Bacterial strains. All strains used in this study were derived from SR108, a *thyA36 deoC2* derivative of W3110. SR108, HL921 [SR108 (*srlR-recA*)306::*Tn*10], HL952 (SR108 *uvrA*::*Tn*10), HL922 (SR108 *recB21C22 argA81*::*tn10*), CL579 (SR108 *recF6206*::Tet^r), HL924 (SR108 *recJ284*::*Tn*10), CL575 (SR108 *umuC122*::*Tn*5), CL634 (SR108 *dinB*::Kan^r), CL636 (SR108 *polB*::ΩSm-Sp), CL646 (SR108 *polB*::ΩSm-Sp *dinB*::Kan^r *umuDC595*::Cam^r), and CL681 (SR108 *polB*::ΩSm-Sp *dinB*::Kan^r *umuDC595*::cat *uvrA*::*Tn*10) all have been reported previously (5, 9, 10).

Survival studies. Overnight cultures were grown in Davis medium supplemented with 0.4% glucose, 0.2% Casamino Acids, and 10 μg/ml thymine (DGCthy medium) (11). Tenfold serial dilutions of these cultures were spotted in triplicate on Luria-Bertani (LB) plates containing 10 μg/ml thymine and nitrofurazone (from TCI America) at the indicated concentrations. For UV irradiation, plates containing spotted dilutions of the cultures were UV irradiated at the indicated doses using a 15-W, 254-nm Sylvania germicidal lamp at an incident dose of 0.9 J/m^2/s. The plates were incubated at 37°C, and the surviving colonies were counted after 48 h for nitrofurazone treatments or overnight for UV irradiation.

Growth curves. Cells (10^5) from fresh overnight cultures were inoculated into a 96-well microtiter plate containing 0, 5, or 10 μM nitrofurazone in DGCthy medium. The 96-well microplate then was incubated at 37°C with constant agitation in an incubating plate reader (model ELX808; Bio Whittaker), and the absorbance at 560 nm was determined at 20-min intervals.

Nitrofurazone-induced mutagenesis. Fresh cultures were diluted 1:100 in DGCthy medium containing 0, 10, or 200 μM nitrofurazone and grown in a shaking, 37°C water bath overnight. Aliquots (200 μl) of each culture were plated on LB plates containing 10 μg/ml thymine and 100 μg/ml rifampin (rifampicin). Ten-μl aliquots of serial dilutions also were spotted in triplicate on LB plates containing 10 μg/ml thymine to determine the number of survivors. The numbers of colonies that grew on rifampin and LB plates were counted after an overnight incubation at 37°C.

Recovery of replication following exposure to nitrofurazone or UV irradiation. Fresh overnight cultures were subcultured 1:100 in DGCthy medium supplemented with 0.1 μCi/ml [^{14}C]thymine and grown in a shaking, 37°C water bath to an optical density at 600 nm of exactly 0.3. The culture then was split equally into two flasks. At this time, half of the subculture was treated with 200 μM nitrofurazone for 20 min, while the other half was mock treated. The cells in each culture then were collected onto Fisherbrand 0.45-μm general membrane filters and resuspended in prewarmed DGCthy medium containing 0.1 μCi/ml [^{14}C]thymine. In the case of UV, half of the subculture received a dose of 27 J/m^2, while the other half was mock irradiated. At the indicated times, duplicate 0.5-ml aliquots were pulse labeled for 2 min with 0.1 μCi/ml [^{3}H]thymidine. The cells then were lysed, the DNA was precipitated and collected, and its radioactivity was determined as described above.

RESULTS

Nucleotide excision repair and the RecBC and RecF pathways are important for cell survival in the presence of nitrofurazone. To examine which repair pathways contribute to the survival of nitrofurazone-induced DNA damage, we compared the survival of various repair mutants to that of wild-type cultures that were grown in the presence of nitrofurazone. Serial dilutions of cultures were spotted on LB plates containing nitrofurazone at various concentrations, and the number of surviving colonies at each concentration was determined. For comparison, the survival of each mutant following exposure to UVC (254 nm) irradiation also was determined. We initially examined the contribution of several genes associated with recombination or replication recovery to survival in the presence of nitrofurazone. We found that similar to the case of UV exposure, cells lacking *recA*, *recBC*, *recF*, and *recJ* were hypersensitive to nitrofurazone relative to the parental strain (Fig. 1A). In almost every case, the relative hypersensitivity of each mutant to nitrofurazone was similar to that seen for UV irradiation. The exception to this was the *recF* pathway. Both *recF* and *recJ* mutants were modestly more sensitive to nitrofurazone than they were to UV irradiation.

We then directly compared the ability of mutants defective in nucleotide excision repair (*uvrA*) to mutants lacking the translesion DNA polymerases (*dinB*, *polB*, and *umuC*) to survive in the presence of nitrofurazone. In separate previous studies, nucleotide excision repair mutants were reported to be resistant to nitrofurazone (21, 27), and mutants lacking translesion Pol IV were found to be more sensitive to nitrofurazone than a strain overexpressing the polymerase (17). Unexpectedly, we observed that *uvrA* mutants were severely sensitive to nitrofurazone, and that their sensitivity was significantly greater than that of the translesion DNA polymerase mutants (Fig. 1B). With respect to the translesion DNA polymerases, we found that only Pol IV (*dinB* gene product) detectably contributed to survival in the presence of nitrofurazone, consistent with previous reports (17). Mutants lacking either Pol V (*umuDC* gene product) or Pol II (*polB* gene product) were as resistant as wild-type cultures at the nitrofurazone concentrations we examined. However, in the absence of all three polymerases, cells were modestly more sensitive than *dinB* mutants alone, raising the possibility that in the absence of Pol IV, Pol

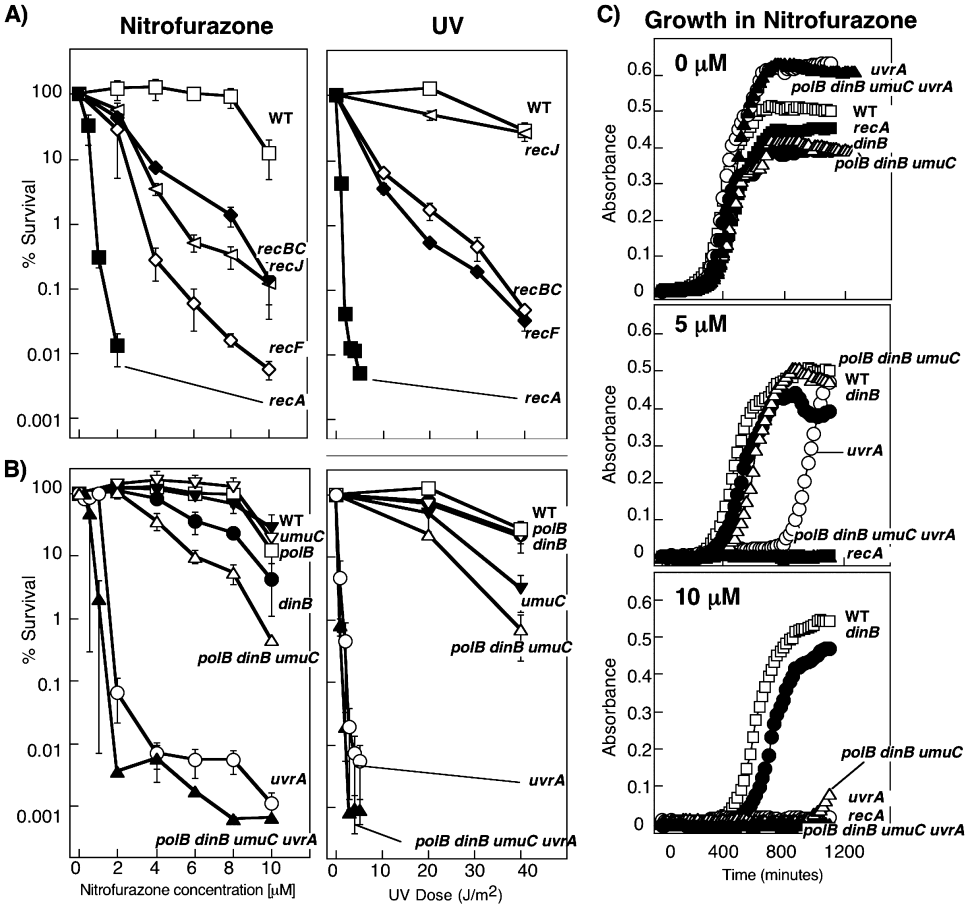

FIG. 1. Nucleotide excision repair and recombination pathways are the predominant mechanisms required for survival in the presence of nitrofurazone-induced DNA damage. (A) The survival of wild-type (WT) (□), *recA* (■), *recBC* (◆), *recF* (◇), and *recJ* (▷) mutants on plates containing nitrofurazone or exposed to UV irradiation at the indicated dose is plotted. (B) The survival of wild-type (□), *uvrA* (○), *dinB* (●), *umuC* (▼), *polB* (▽), *polB dinB umuC* (△), and *polB dinB umuC uvrA* (▲) mutants is plotted. Error bars represent the standard errors of the means from at least three independent experiments. (C) Growth, as measured by absorbance at 560 nm, of wild-type (□), *recA* (■), *uvrA* (○), *dinB* (●), *polB dinB umuC* (△), and *polB dinB umuC uvrA* (▲) cultures in the presence of 0, 5, or 10 μM nitrofurazone is plotted over time. Cultures were inoculated with 10^5 cells/ml as indicated. Graphs represent one of two individual experiments.

II or Pol V also participates in processing nitrofurazone-induced damage.

In an alternative approach, we also monitored the growth of cultures in media containing nitrofurazone. To this end, 10^5 cells from fresh overnight cultures were inoculated in media containing 0, 5, or 10 μM nitrofurazone. The relative growth of each culture then was measured by the change in absorbance of 560-nm light over time. In the case of all mutants examined, the extent of the growth delay due to nitrofurazone correlated with their relative sensitivity on LB agar plates containing nitrofurazone (Fig. 1C). Interestingly, the effect of nitrofurazone on growth in liquid culture was associated with a prolonged lag phase rather than an overall increase in the doubling time of the growing cultures. Once visible growth had begun, cultures grown in the presence of nitrofurazone grew as quickly as cultures grown in the absence of nitrofurazone, including those mutants that were hypersensitive to nitrofurazone, such as *uvrA*.

Taken together, we interpret these results to indicate that nucleotide excision repair is a prominent mechanism for surviving nitrofurazone-induced DNA damage in vivo. In early studies that found that *uvrA* mutants were not hypersensitive to nitrofurazone, cultures were exposed to high drug concentrations (50 to 250 μm) for a period of 20 min (20). When we limited the exposure of cultures to nitrofurazone for 20 min or less, we also observed that the hypersensitivity of *uvrA* mutants was largely diminished, consistent with the earlier studies (see Fig. 3 and data not shown). The lack of hypersensitivity in *uvrA* following acute high-dose exposures can be accounted for by the antimicrobial properties of the drug when used at high concentrations and the relatively low rate at which DNA lesions are induced by this agent (see below).

Loss of nucleotide excision repair, but not translesion synthesis, increases mutagenesis induced by nitrofurazone. Although the presence or absence of translesion DNA polymerases often does not affect cell survival, their effect on mutagenesis can be comparatively dramatic. Early studies found that nitrofurazone is a weak, *recA*-dependent mutagen in *E. coli* and suggested that the mutagenesis is mediated by the error-prone repair system, which now is considered to represent the translesion DNA polymerases (27). However, a subsequent study of *Salmonella enterica* serovar Typhimurium

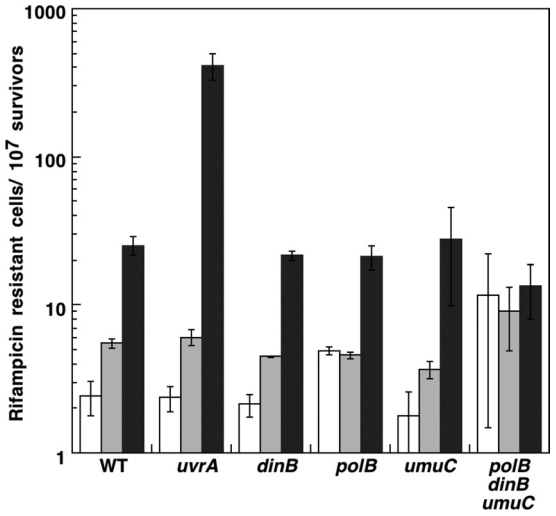

FIG. 2. Inactivation of nucleotide excision repair, but not translesion synthesis, increases the frequency of rifampin-resistant mutations induced by nitrofurazone. Nitrofurazone was added to exponentially growing cultures before the cultures were plated to determine the frequency of rifampin-resistant cells in the culture. □, 0 μM nitrofurazone; ▥, 10 μM nitrofurazone; ■, 200 μM nitrofurazone. Error bars represent the standard errors of the means from two independent experiments. WT, wild type.

found that nitrofurazone did not induce mutations, and a more recent study found that Pol IV could efficiently bypass N^2-dG adducts generated by nitrofurazone with high fidelity (17, 28). Thus, to determine if nitrofurazone is mutagenic and to identify the polymerase(s) responsible for replication errors at nitrofurazone-induced damage, we examined the frequency of rifampin resistance in cultures after exposure to nitrofurazone in both nucleotide excision repair and translesion polymerase mutants. Resistance to rifampin can arise from 1 of at least 69 base substitutions within the *rpoB* gene, allowing one to monitor numerous mutation sites in different sequence contexts (13). In wild-type cultures, we observed that the number of rifampin-resistant colonies increased modestly as a function of nitrofurazone dose, consistent with the idea that nitrofurazone is a weak mutagen (Fig. 2). A similar increase in the frequency of rifampin-resistant colonies was observed in the absence of Pol II, Pol IV, Pol V, or all three translesion DNA polymerases, suggesting that no individual polymerase is responsible for the mutations induced by nitrofurazone. In *uvrA* mutants, we observed that rifampin-resistant colonies increased roughly 10-fold compared to wild-type cultures or translesion DNA polymerase mutants. The observation is consistent with previous studies that found that the bypass of nitrofurazone-induced N^2-dG adducts by Pol IV preferentially incorporates cytosine and does not result in mutation. These results also support the idea that nucleotide excision repair plays a prominent role in removing nitrofurazone-induced DNA damage and indicates that the absence of lesion removal reduces both survival and replication fidelity.

High concentrations of nitrofurazone inhibit DNA synthesis independently from nitrofurazone-induced DNA damage. We next wanted to determine the mechanism by which the cell processes and recovers replication following nitrofurazone-induced DNA damage compared to that following UV-induced damage. To address this question, we measured the rate of DNA synthesis following exposure to either UV or nitrofurazone by incubating [^{14}C]thymine-prelabeled cultures for 2 min with [^{3}H]thymidine at various times after treatment. The rate of DNA synthesis, as measured by ^{3}H incorporation in the DNA, then could be determined relative to the total amount of ^{14}C-labeled DNA present at specific times following treatment. Exposures of 25 J/m^2 of UV and 20 min of 200 μM nitrofurazone were selected, because we found that these doses would inhibit DNA synthesis in cultures by more than 90% (Fig. 3 and data not shown). Since the rate of DNA synthesis varies significantly with cell density, all experiments included a mock-treated control that allowed us to directly compare exposed and unexposed cultures and ensure that any observed differences were due to nitrofurazone or UV treatment rather than culture density.

As demonstrated previously for UV and shown in Fig. 3A for the purpose of controls, the rate of DNA synthesis in wild-type cultures began to recover approximately 15 min after UV irradiation and had almost completely recovered to a rate comparable to that of unirradiated controls 90 min after treatment. By comparison, in mutants lacking RecF, which is required with RecA to maintain replication forks arrested at DNA damage, the rate of DNA synthesis does not recover, and no further DNA accumulation is observed (7, 8, 10). In previous work, we have shown that DNA synthesis recovers at a time that correlates with the removal of the lesions by nucleotide excision repair. In *uvrA* mutants that cannot remove the blocking lesions, no further synthesis or accumulation of DNA is observed (5–7, 10).

When we examined the recovery of DNA synthesis in wild-type cultures treated with nitrofurazone, we observed an inhibition in DNA replication similar to that in UV-irradiated cultures. However, replication resumed more rapidly after nitrofurazone treatment than after UV irradiation (Fig. 3B). In wild-type cultures, DNA synthesis began to recover as soon as the drug was removed and had fully recovered to a rate comparable to that of untreated cultures within 30 min. Surprisingly, in *recF* mutants, which are unable to recover replication following disruption by DNA damage, we observed a similarly rapid and full recovery of DNA synthesis once the drug was removed. Curiously, the rate of DNA synthesis also recovered rapidly in several other mutants that were shown to be hypersensitive to nitrofurazone in the growth media, including *uvrA*, *recBC*, and a mutant lacking all three translesion DNA polymerases. Previous studies have shown that in *recA* mutants, a single replicational encounter with DNA damage disrupts replication and is lethal (16). However, even in *recA* mutants, we observed that DNA synthesis began to recover with kinetics similar to that of wild-type cultures, although in this case the initial rapid recovery in the rate of DNA synthesis gradually slowed, and after 30 min the rate began to decline again (Fig. 3B). To further characterize the recovery that occurred under these conditions, we also measured the survival of cultures treated in this manner. We found that the hypersensitive phenotype exhibited by *uvrA*, *recF*, and *recBC* mutants, when grown in the presence of low nitrofurazone concentrations (Fig. 1), was largely diminished when the exposure was limited to 20 min at high drug concentrations (Fig. 3C).

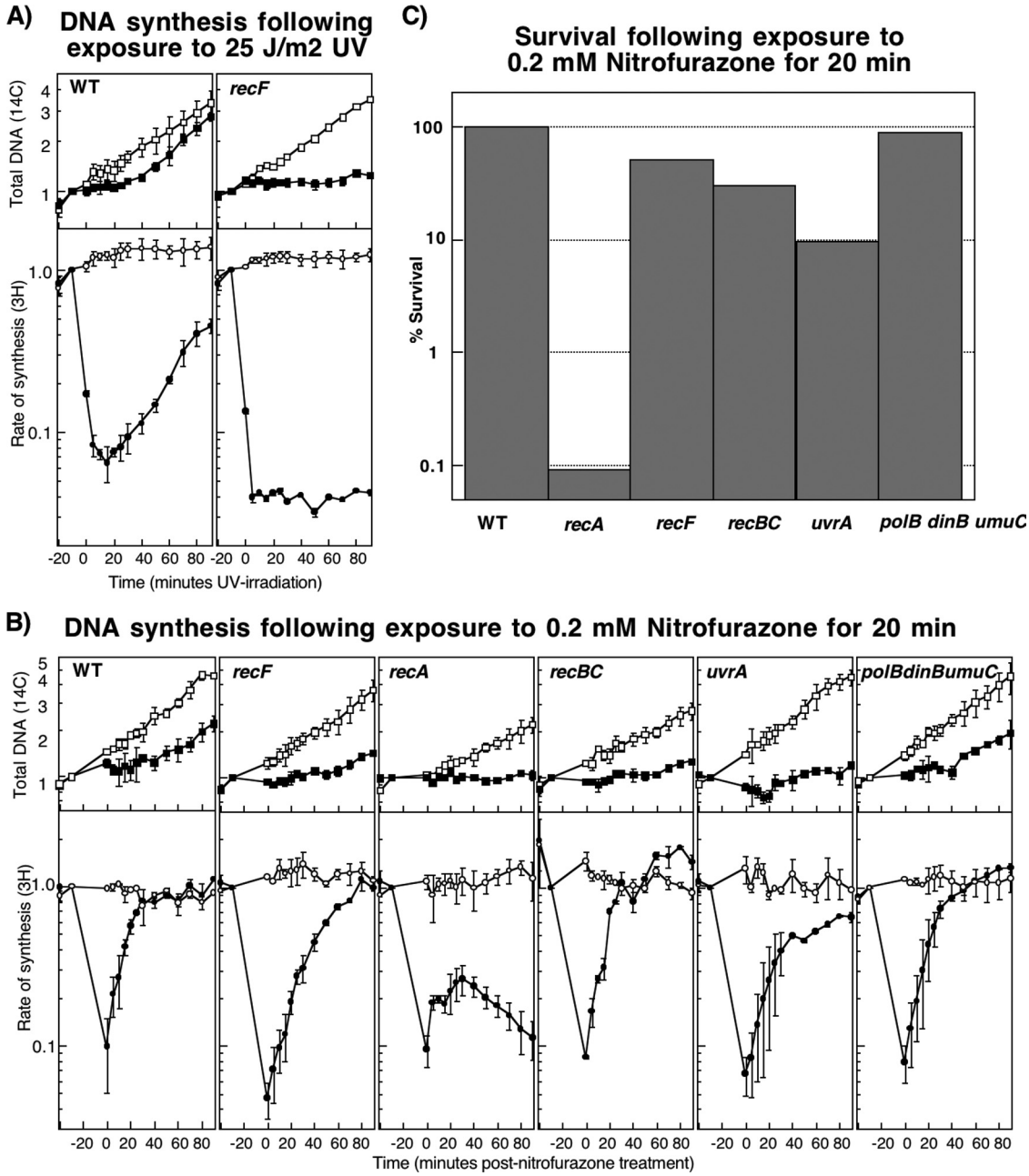

FIG. 3. High concentrations of nitrofurazone inhibit DNA synthesis directly, independently of DNA damage. (A) Cultures grown in [^{14}C]thymine were treated with 200 μM for 20 min before cells were collected on filters and resuspended in fresh drug-free media. At the indicated times, aliquots of the culture were pulsed with [^{3}H]thymidine for 2 min. The amount of ^{3}H and ^{14}C incorporated in the DNA at each time point is plotted relative to the amount incorporated just prior to nitrofurazone addition. □, ^{14}C mock treated; ■, ^{14}C treated; ○, ^{3}H mock treated; ●, ^{3}H treated. Error bars represent the standard errors from two independent experiments. WT, wild type. (B) The same as described for panel A, except that cells were UV irradiated with 27 J/m^2. (C) The percent survival of each culture following a 20-min exposure to 200 μM nitrofurazone is plotted. Error bars represent the standard errors of the means from two independent experiments.

The observed recovery and survival of *uvrA* mutants strongly suggests that the 200 μM nitrofurazone treatment inhibits DNA replication directly, independently of DNA damage. Consistent with this interpretation, *recF* and, initially, *recA* mutants that fail to resume DNA synthesis following disruption by DNA damage also were able to recover replication once the nitrofurazone was removed. In *recA* mutants, the ultimate cessation of replication that occurs 30 min after replication initially recovers likely is due to low levels of nitrofurazone-induced DNA damage that eventually are encountered by the replication machinery (see below). However, the direct inhibition of replication caused by nitrofurazone is reversible, since replication recovered immediately after the drug is removed in all mutants that were examined.

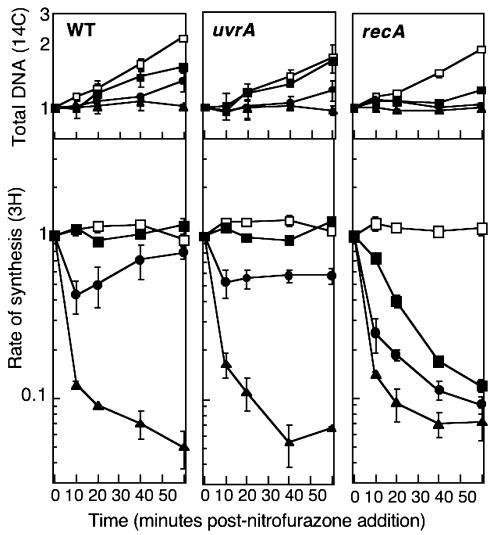

FIG. 4. Prolonged nitrofurazone exposure is required to induce DNA damage at levels sufficient to disrupt DNA replication. Cultures grown in [^{14}C]thymine-containing media were pulse labeled with [^{3}H]thymidine for 2 min at specific times after nitrofurazone was added to the media. The amount of ^{3}H and ^{14}C incorporated into the DNA is plotted relative to the amount incorporated just prior to the addition of nitrofurazone. □, 0 μM nitrofurazone; ■, 25 μM nitrofurazone; ●, 100 μM nitrofurazone; ▲, 200 μM nitrofurazone. Error bars represent the standard errors of the means from at least two independent experiments. WT, wild type.

When lower, subinhibitory concentrations of nitrofurazone were used, we found that replication could continue, but that DNA damage was induced at a rate that was too low to synchronously arrest replication in the culture and allow us to monitor the mechanism of recovery using this type of approach (Fig. 4). To examine the effect that lower nitrofurazone concentrations had on replication, we pulse-labeled aliquots of cultures with [^{3}H]thymidine as before to monitor the rate of DNA synthesis at specific times after the addition of 0, 25, 100, or 200 μM nitrofurazone. By this assay, we observed that DNA synthesis continued during the entire 90-min assay in both wild-type and *uvrA* mutant cultures when 25 or 100 μM nitrofurazone was present in the media. Further, in *recA* cultures, the rate of replication declined only gradually at these concentrations, consistent with the previous assay and supporting the idea that the level of DNA damage induced under these conditions is relatively low. Taken together, Fig. 3 and 4 strongly suggest that the inhibition of replication by high concentrations of nitrofurazone occurs independently of DNA damage.

DISCUSSION

The results presented here demonstrate that survival following DNA damage induced by nitrofurazone predominantly depends on nucleotide excision repair rather than translesion synthesis. In addition, nitrofurazone survival also depends on both the RecF and RecBC pathways, similar to UV-induced damage. The similarity in genes required for surviving nitrofurazone and UV-induced damage strongly suggests that the cell processes both types of lesions and recovers replication through similar pathways. However, the low frequency and rate at which DNA damage is induced by nitrofurazone prevented us from measuring the recovery of replication directly in these mutants. With respect to translesion synthesis and consistent with previous studies, we observed that Pol IV contributes to survival after nitrofurazone treatment, whereas Pol V contributes to survival after UV-induced damage.

The cellular role of the translesion DNA polymerases has proven difficult to determine. We initially chose to characterize nitrofurazone-induced DNA damage, because we felt that a number of biochemical and genetic studies suggested that translesion synthesis played a more prominent role in promoting survival than UV-induced damage (12, 21, 27, 46). Our results indicate that the role of translesion DNA polymerases in processing nitrofurazone-induced DNA damage is similar to that seen with other forms of DNA damage. In both *E. coli* and yeast, the presence or absence of these polymerases generally does not have large effects on viability, nor does it prevent the replication of the genome from occurring when DNA damage is present (5, 6, 41). Although the effect that translesion polymerases have on viability is relatively minor, Pol II, Pol IV, and Pol V each have been shown to affect mutation frequencies when challenged with specific forms of DNA damage (18, 31). This correlation appears to extend to human cells as well. Human patients with the variant form of Xeroderma pigmentosum lack translesion polymerase η, which efficiently bypasses UV-induced lesions (22). Although cells from these patients are as susceptible to mutations and cancer as the repair-deficient forms of Xeroderma pigmentosum, they are not significantly hypersensitive to UV-induced DNA damage (4). Taken together, these observations imply that the cellular role for these polymerases is not related to restoring replication following disruption by DNA damage under normal conditions.

A prominent role for nucleotide excision repair after nitrofurazone-induced DNA damage contrasts with previous studies that found that nucleotide excision repair mutants were not hypersensitive to nitrofurazone (21, 27). These previous studies utilized high concentrations of nitrofurazone in cultures with short (20 min) exposure times. In this study, we showed that these conditions inhibit replication but are not sufficient in duration to induce significant levels of DNA damage. Longer exposure times may be required before the nitrofurazone can be activated by the cellular nitroreductases. Alternatively, the permeability of the drug across the cell membrane also may be a limiting factor in the rate at which DNA damage is induced.

Nitrofurazone often is used as a topical antimicrobial, yet its mechanism of action remains unclear. In this study, we demonstrate that nitrofurazone directly and reversibly inhibits DNA synthesis, suggesting a potential mode of action. Although nitrofurazone has not been reported previously to inhibit DNA synthesis directly, other nitrofuran derivatives are known to be specific inhibitors of replication in *E. coli* (26). We demonstrate that the inhibition is not due to DNA lesions that block the replication machinery, since mutants known to be defective in restoring DNA synthesis after disruption by DNA damage (*recF*) and mutants that are hypersensitive to nitrofurazone-induced DNA damage (*uvrA*) can resume DNA synthesis once the nitrofurazone is removed from the media. Taken together, these results demonstrate that nitrofurazone toxicity is mediated by two distinct mechanisms. Based on the genes required for growth in the presence of nitrofurazone, it

generates DNA lesions that can disrupt replication and cause lethality if left unrepaired. In addition, high concentrations of nitrofurazone can inhibit DNA replication directly and prevent cell growth.

ACKNOWLEDGMENTS

We thank S. Estes for helpful comments on the manuscript.

This work was supported by CAREER award MCB0551798 from the National Science Foundation and AREA grant R15GM86839 from the National Institutes of Health.

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