SOS-Independent Induction of *dinB* Transcription by β-Lactam-Mediated Inhibition of Cell Wall Synthesis in *Escherichia coli*

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Transcription of the *dinB* gene, encoding DNA polymerase IV, is induced by the inhibition of cell wall synthesis at different levels. Using the β-lactam antibiotic ceftazidime, a PBP3 inhibitor, as a model, we have shown that this induction is independent of the LexA/RecA regulatory system. Induction of *dinB* transcription mediated by ceftazidime produces an increase in the reversion of a +1 Lac frameshift mutation.

The *Escherichia coli* SOS system comprises at least 30 different genes, some of them encoding proteins involved in DNA repair pathways (7). When activated by single-strand DNA (which can be created by multiple events, such as DNA damage, stalled replication, and conjugation, etc.), the coprotease activity of the RecA protein promotes the self-cleavage of the LexA protein (the SOS repressor) and, consequently, induces the SOS response (3). Among the known SOS genes are *polB*, *dinB*, and *umuDC*, encoding the specialized DNA polymerases II, IV, and V (Pol II, Pol IV, and Pol V), respectively (8). These enzymes are able to bypass DNA lesions that block chain elongation by the replicative DNA polymerase. When operating on nonsubstrate templates or copy-noncognate lesions, these enzymes exhibit reduced fidelity and, consequently, produce mutations in the newly synthesized DNA strand (8, 19). Thus, as a consequence of DNA damage or a cessation of DNA replication, the mutation rate of bacteria is increased (17). Finally, the importance of these enzymes for the long-term survival of *E. coli* has been demonstrated (23).

The activity of Pol IV is involved in adaptive mutation (21). Overproduction of Pol IV produces a mutator phenotype (10). Thus, its synthesis has to be regulated in normal (nonstressed) cells to keep its mutagenic activity under control. It has recently been described that, in addition to DNA damage or DNA replication, other types of stress, including stationary phase, can trigger the transcription of *dinB* (11). This SOS-independent regulation indicates that induction of Pol IV is part of a more general stress-regulated response. This result has suggested to some researchers that DNA polymerase IV may be part of a mechanism to produce mutants when the population is under extreme conditions (4, 11, 13).

There are a large number of situations that can produce stress in bacteria. Some of them, such as the presence of

antibiotics, are specifically used to produce different types of vital stress in bacteria and to eliminate them from humans. Thus, it is tempting to test whether different antibiotics, as stress producers, can potentially induce *dinB* transcription and as a result increase the mutation frequency.

It was previously shown that some antibiotics used in clinical practice, such as quinolones, are good inducers of the SOS system and, consequently, increase the mutation frequency (16). This increase in mutation frequency was shown to be due to the activity of Pol V (24). Also, streptomycin, an aminoglycoside antibiotic, is known to promote mistranslation and induce a *recA*- and *umuDC*-independent mutator phenotype (18).

In this work, we have investigated the capacity of different antibiotic families to induce the transcription of the *dinB* gene and to increase the rate at which mutations are produced. Our results show that some antibiotics, known to act as cell wall synthesis inhibitors at different steps but without having any effect on DNA (damage or replication) or translation are able to induce *dinB* transcription. Using the β-lactam antibiotic ceftazidime (CAZ), which is a widely used PBP3 inhibitor, as a model, we have demonstrated that this induction occurs independently of the LexA/RecA regulators. In addition, we have shown that CAZ can increase, although slightly, the reversion frequency of a +1 Lac frameshift mutation in a *dinB*-dependent way.

Antibiotics that induce *dinB* transcription. Using a disk plate assay, we tested the capacity of different antibiotic families to induce *dinB* transcription. Aliquots of 100 μl from overnight cultures of strain GW1030 [*dinB1*::MudI (Ap *lac*)], harboring a *dinB*::*lacZ* operon fusion (9), were resuspended in soft Luria-Bertani (LB) agar, mixed, and plated onto LB plates containing 50 μg of X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside)/ml. Disks containing different antibiotics were put onto the seeded bacteria, and the plates were incubated for 24 h at 30°C. We found that, as expected, quinolones, such as ciprofloxacin (Fig. 1A) and nalidixic acid (NAL) and norfloxacin (results not shown), produced a strong blue band
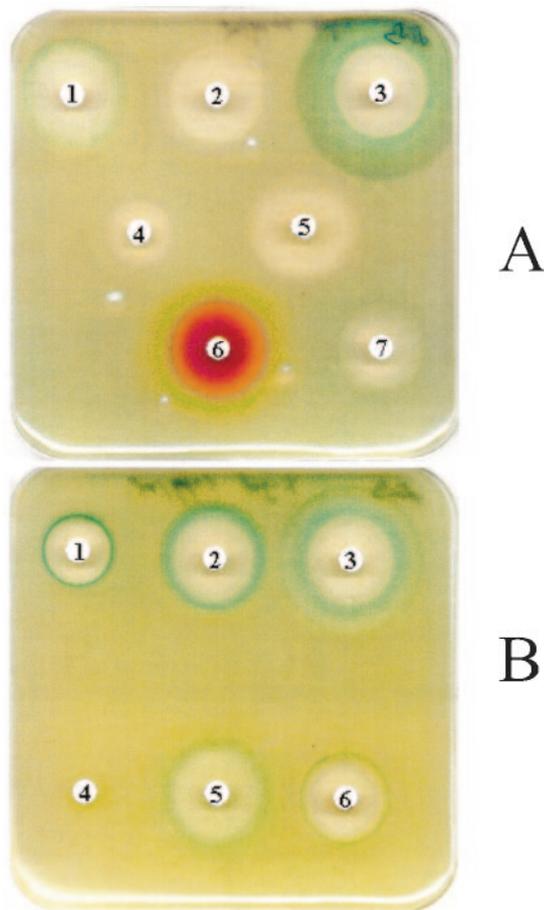

FIG. 1. Disk plate assays showing the effects of different antibiotics on the transcription of the *dinB*::*lacZ* operon fusion. (A) Antibiotics belonging to different families (the charge of each disk [in micrograms] is in parentheses) were as follows: on disk 1, ceftazidime (30); on disk 2, chloramphenicol (50); on disk 3, ciprofloxacin (5); on disk 4, erythromycin (30); on disk 5, tetracycline (50); on disk 6, rifampin (100); and on disk 7, amikacin (30). (B) Antibiotics affecting the cell wall synthesis at different steps (the charge of each disk [in micrograms] is in parentheses) were as follows: on disk 1, amoxicillin-clavulanate (30); on disk 2, imipenem (10); on disk 3, fosfomycin (50); on disk 4, no antibiotic; on disk 5, aztreonam (30); and on disk 6, D-cycloserine (100). All disks were purchased from Oxoid Ltd., except those containing rifampin and D-cycloserine, which were prepared in our laboratory.

in the border of the inhibition halo, reflecting their SOS induction capacity. In contrast, other structurally unrelated antibiotics, such as tetracycline, amikacin, rifampin, erythromycin, and chloramphenicol, did not show any apparent induction (Fig. 1A). However, different β-lactam antibiotics, such as ceftazidime (Fig. 1A), aztreonam, and imipenem (Fig. 1B), also induced the transcription of the *dinB*::*lacZ* operon fusion, as deduced from the blue band in the border of the inhibition halo.

The results obtained with β-lactam antibiotics suggested that cell wall damage may affect *dinB* transcription. Consequently, we tested the effects of several antibiotics known to affect different steps in cell wall physiology. Results shown in Fig. 1B indicate that the inhibition of cell wall biosynthesis (via fosfo-

mycin or D-cycloserin), elongation by inhibition of PBP2 (via imipenem), or septation by inhibition of PBP3 (via aztreonam) induces *dinB* transcription. These results indicate that, independently of the level at which it is produced, cell wall synthesis inhibition leads to the induction of *dinB* transcription.

CAZ-mediated *dinB* induction is independent of LexA and RecA. To understand better the molecular mechanism involved in *dinB* induction, we examined *dinB* expression using the β-lactam antibiotic CAZ as a model. As a part of the SOS regulon, *dinB* transcription is regulated by LexA and RecA. Thus, we tested the induction of the *dinB*::*lacZ* fusion in *recA*-defective and *lexA1* Ind⁻ backgrounds. Liquid cultures of strain GW1030 and its derivatives GW1030-*recA* Δ(*srlR-recA*)306::Tn*10* and GW1030-*lexA1* (*lexA1 malE*::Tn*5*), constructed by P1 transduction (14), were exposed to many different CAZ concentrations around the MIC, which, under our experimental conditions (i.e., LB agar, a large amount of inoculum, and 30°C), was determined to be 1.0 μg/ml. The β-galactosidase levels were determined after different exposure times (14). Figure 2A shows that the *dinB* transcription induction mediated by ceftazidime is time and concentration dependent in the wild-type context. Surprisingly, CAZ was able to induce the transcription of the *dinB*::*lacZ* fusion when SOS induction is blocked (i.e., in Δ*recA* or *lexA1* backgrounds), although the relative values of induction in the Δ*recA* context are lower than in both the wild-type and *lexA1* backgrounds, indicating that the effect may be not fully *recA* independent. The same experiments were performed in parallel using nalidixic acid as the inducer. In this case (Fig. 2B), *dinB*::*lacZ* transcription was induced in strain GW1030 but not in its Δ*recA* or *lexA1* derivatives.

CAZ produces an increase in the frequency of Lac⁺ revertants of strain SMR4562 that is mediated by DNA polymerase IV. Because overproduction of DNA polymerase IV causes a mutator phenotype (10, 22), it was possible that the CAZ-mediated increase in *dinB* transcription could also cause an increase in the mutation frequency. As CAZ produces filamentous multinucleated cells when it inhibits its PBP3 target, the classical method for calculating the mutation frequency may lead to an overestimation of this frequency. To avoid this potential problem, we modified the classical experiment described by Cairns and Foster (5). About 10^8 SMR4562 cells carrying the allele *lac* +1 frameshift in the F128 episome {Δ(*lac-proAB*) *thi ara* [F′ *proAB lacI33ΩlacZ*]} (12) or SMR5830 cells (*dinB10* [F′ *dinB10 proAB⁺ lacI33ΩlacZ*]) (13) from overnight LB agar stationary-phase cultures were resuspended in 4 ml of soft minimal agar and plated onto minimal M9 plates containing lactose as the only carbon source. The plates were incubated for 2 h at 37°C, and then soft agar containing appropriate quantities of CAZ was added to the plates. By this procedure, we ensured that every Lac⁺ colony was derived from a single plated cell, independently of the number of nucleoids present per filamented cell after the CAZ challenge. The numbers of Lac⁺ colonies and of viable cells were determined before and 1 and 2 days after the CAZ treatment for both treated and untreated cells. The viable counts (obtained by taking plugs from the lactose plates) were similar for control plates and for plates containing 0.005, 0.01, or 0.02 μg of CAZ/ml. Plates with 0.04 μg of CAZ/ml showed small decreases in viable counts, and those with higher con-
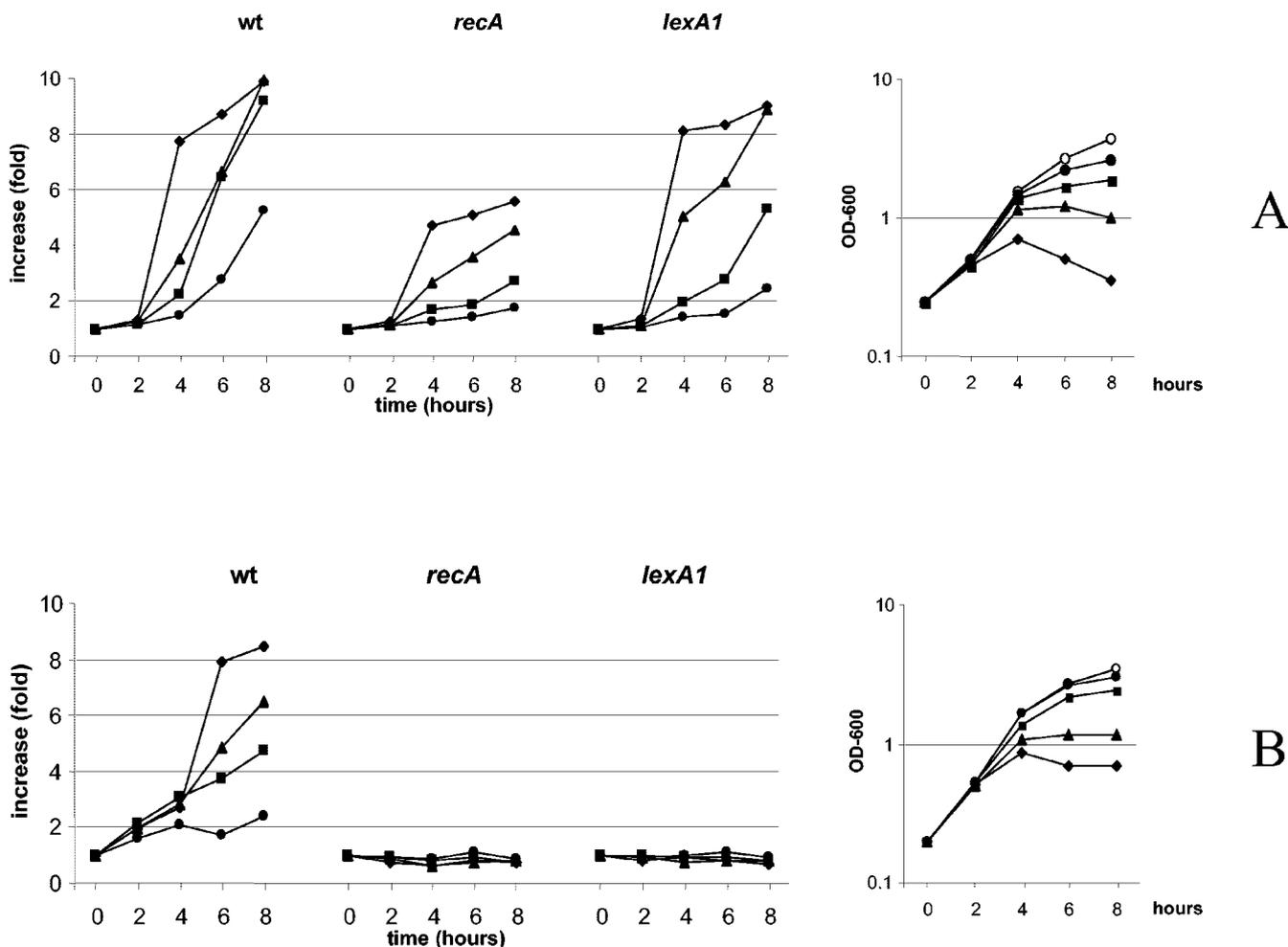
FIG. 2. Effects of the Δ(*srlR-recA*)306::Tn*10* and *lexA1* genetic backgrounds on the ceftazidime-mediated induction of transcription of the *dinB*::*lacZ* fusion. The curves on the left of each panel indicate the transcription levels (*n*-fold) between treated and nontreated cultures (calculated by assigning a value of 1 to the transcription level of the nontreated culture at each time point). Concentrations of CAZ (A) and NAL (B) expressed in micrograms per milliliter were as follows: 1 (◆), 2 (▲), 4 (■), and 8 (●). The mean values of results from three independent experiments are displayed. The curves on the right of each panel represent the growth curves without and with CAZ or NAL for strain GW1030 (symbols are as described for the transcription levels; ○ indicates the values obtained without CAZ). Curves for *recA* and *lexA1* derivatives are not presented because they are similar to those for strain GW1030. OD-600, optical density at 600 nm; wt, wild type.

centrations of CAZ showed clear decreases in the number of viable cells at concentrations above 0.08 μg/ml. This result is consistent with the MIC of CAZ in minimal M9 medium for strains SMR4562 and SMR5830 (0.1 to 0.2 μg/ml). Ten independent experiments were carried out with strain SMR4562 and its *dinB10* derivative. Mutant frequencies were calculated as the mean number of Lac$^+$ colonies per 10^8 plated cells for CAZ-treated and nontreated SMR4562 and its *dinB10* derivative 3 days after CAZ addition. Figure 3 shows that CAZ concentrations of 0.005, 0.01, and 0.02 μg/ml produced small increases in the rate of Lac reversion in strain SMR4562, with a maximum increase at 0.02 μg/ml. As stated above, at concentrations above 0.04 μg of CAZ/ml, cell viability is reduced and, even if the increase in *dinB* transcription is proportional to the CAZ concentration, the number of Lac$^+$ colonies is below the limit of detection under our experimental conditions. Contrary to what happens with strain SMR4562, CAZ addition does not increase the frequency of Lac reversion in
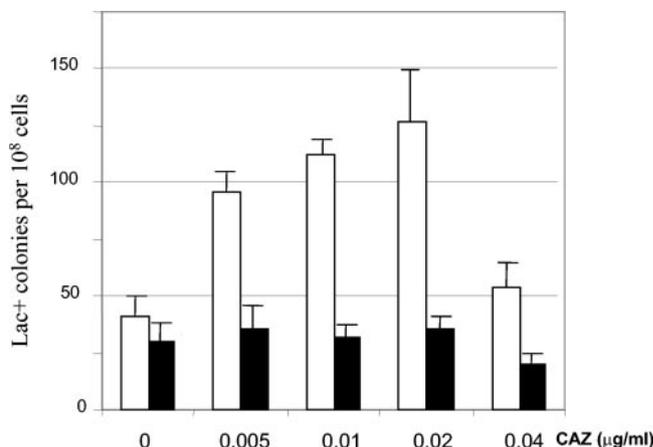

FIG. 3. Number of Lac$^+$ revertants after CAZ treatment. Bars represent the mean numbers of Lac$^+$ colonies per 10^8 plated cells of CAZ-treated and nontreated SMR4562 (open bars) and its *dinB10* derivative (filled bars) after 3 days of incubation. T bars represent standard deviations.

the strain lacking *dinB*, SMR5830 *dinB10*, indicating that this increase is *dinB* dependent.

There is evidence of interactions between the SOS response and other stress response pathways. In *E. coli*, induction of some SOS genes may occur as a function of growth phase (1, 6) or in resting bacteria in structured habitats (20). The stationary-phase sigma factor RpoS positively controls transcription of the *dinB* gene in *E. coli* (11). Thus, one can speculate that inhibition of cell wall synthesis by CAZ may cause an RpoS-dependent *dinB* response. To test this hypothesis, we studied the induction of the *dinB::lacZ* fusion in an *rpoS*-defective derivative of GW1030 (constructed by P1 transduction). Induction of *dinB* transcription occurred also in the *rpoS*-deficient background (data not shown). Thus, the induction of *dinB* transcription produced by inhibition of cell wall synthesis seems to be independent of RpoS.

It was previously shown that chromosome replication stop induces a septation arrest through induction of transcription of the SOS gene *sulA* (15). However, to our knowledge, this is the first time that induction of a DNA polymerase of the SOS system, due to a stop in cell wall synthesis, is described. Experiments are being performed in our laboratory to decipher the molecular basis of the induction of *dinB* transcription by cell wall synthesis inhibition.

Whether this increase in mutation frequency produced by β-lactam treatment, mediated by DNA polymerase IV, is important for the acquisition of resistance mutations in nature remains to be demonstrated. However, the results presented here are of particular concern. The paradoxical possibility that antibiotic treatments increase the rate of antibiotic resistance acquisition exists (2).

ADDENDUM IN PROOF

A recent article by Miller et al. (C. Miller, L. E. Thomsen, C. Gaggero, R. Mosseri, H. Ingmer, and S. N. Cohen, Science, **305:**1629–1631, 2004) provided evidence that PBP-3 inhibition resulted in the induction of the SOS response in *E. coli*. Although part of the results presented here are similar, our study gives different results in some respects. (i) Miller et al. described that the induction of SOS mediated by β-lactams required RecA and LexA. Here we demonstrate that the induction of *dinB* is independent of RecA and LexA. (ii) Whereas Miller et al. reported that the SOS induction was exclusively produced by inhibition of cell septation (PBP3 inhibition), we demonstrate that *dinB* transcription is induced by inhibition of cell wall biosynthesis at different steps.

Our results suggest the existence of an additional SOS-independent pathway for the induction of *dinB* mediated by the inhibition of the cell wall synthesis.

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