JOURNAL OF BACTERIOLOGY, June 1995, p. 3610–3612
0021-9193/95/$04.00+0

Vol. 177, No. 12

SOS Induction in *Escherichia coli* by Single-Stranded DNA of Mutant Filamentous Phage: Monitoring by Cleavage of LexA Repressor

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Received 14 November 1994/Accepted 11 March 1995

Infection of *Escherichia coli* in the presence of chloramphenicol with mutant filamentous phage that are defective in the initiation of minus-strand DNA synthesis induces the SOS response as monitored by cellular LexA levels. This observation demonstrates that single-stranded DNA serves as a primary signal for SOS induction in vivo.

DNA damage in *Escherichia coli* results in the induction of the SOS genes, many of which are involved in DNA repair. The induction is due to cleavage of the LexA protein, the repressor of the SOS genes, by the action of activated RecA protein. Damage to DNA leads to the production of an inducing signal, which in turn activates the RecA protein (for a review, see reference 18). In vitro studies have revealed that RecA can be activated by forming a ternary complex with single-stranded DNA (ssDNA) and a nucleoside triphosphate (1, 2). However, the exact nature of the signal for the SOS induction in vivo has been controversial. There has been no direct in vivo evidence that ssDNA by itself can induce the SOS functions.

We have previously reported that the SOS response is induced by infection with R377, a mutant of the filamentous, ssDNA phage f1 that is defective in initiation of complementary (minus)-strand synthesis. Infection with wild-type f1 did not induce the SOS response. On the basis of this observation we proposed that ssDNA is the primary signal of SOS induction in vivo (7), since the parental DNA of the mutant phage remained in a single-stranded form for a long period after infection. Gratuitous SOS induction by rolling-circle-type replication of a plasmid has also been reported (3). However, since R377 was still able to synthesize the double-stranded replicative form to some extent and to eventually produce progeny phage, although at a low level, and since the SOS induction was monitored by expression of SOS genes in a time range of a few hours, it was not impossible that the SOS response was induced by some other factor(s) that might be produced by the abnormal, slow infection cycle. To rule out such a possibility, we show in this paper that the SOS response is induced by R377 in the presence of chloramphenicol, which inhibits all phage-specific syntheses except for the conversion of parental ssDNA to a double-stranded form (for a review on filamentous phage replication, see reference 15).

SOS response in the presence of chloramphenicol. In this study, we carried out the phage infection in the presence of chloramphenicol and monitored the SOS induction by the decrease in the level of LexA repressor, which represents the first step of the SOS response. The LexA level was quantitatively measured by Western blotting (immunoblotting) with anti-LexA antibody (17). *E. coli* K38 (HfrC λ *phoA6 tonA22 garB10 ompF627 relA1 pit-10 spoT1* $T2^r$ *PO2A*) (7) cells were freshly grown to 10^8/ml at 37°C in the TY medium (19), and chloramphenicol was added to 100 μg/ml. Ten minutes later, the cultures were shifted to 34°C, and 5×10^9 PFU of wild-type or R377 mutant phage per ml was added (time zero). Aliquots (1 ml) were harvested at several time points, spun, and

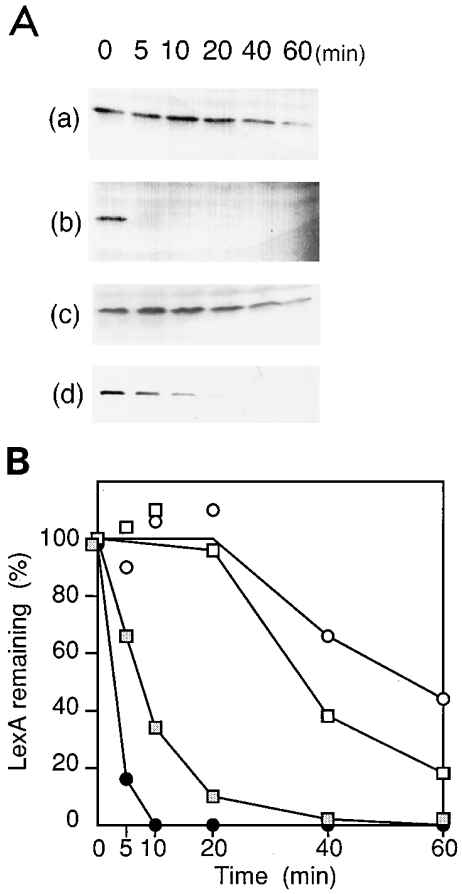

FIG. 1. Decrease in LexA level in *E. coli* cells infected with R377, a mutant filamentous phage that is defective in minus-strand DNA replication. The experiment was carried out in the presence of chloramphenicol (100 μg/ml), which was added 10 min prior to phage addition or UV irradiation. (A) Western blotting of LexA. (a) Control culture (K38 cells); (b) UV-irradiated cells ($7J/m^2$); (c) cells infected with wild-type f1 phage; and (d) cells infected with R377. At the times indicated, cells were harvested and LexA was immunochemically detected after Western blotting as described in the text. (B) Quantification of the data shown in panel A. Open circles, control culture; closed circles, UV-irradiated cells; open squares, cells infected with wild-type f1; shaded squares, cells infected with R377.

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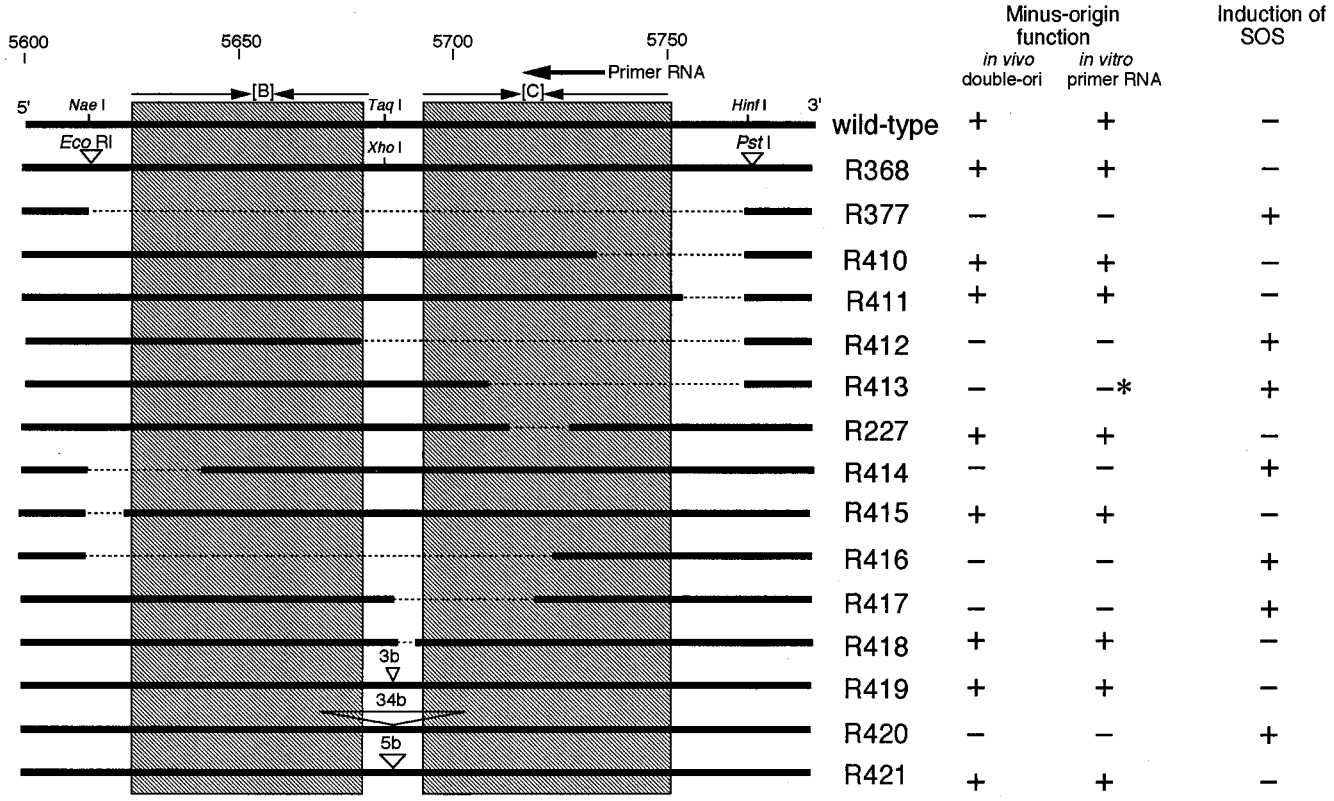

FIG. 2. Correlation between inducibility of the SOS response and the defectiveness in the minus-strand-origin function in various mutants of phage f1. The left part of the figure shows the regions of deletion and insertion in various mutants. The numerals on the top are nucleotide numbers (8). The thick arrow indicates the position of the primer RNA (5). Thin arrows show inverted repeats that form two hairpin structures ([B] and [C]; shaded areas) in the wild-type sequence. Dotted lines indicate the sequence deleted in each mutant. Triangles marked 3b, 34b, and 5b indicate insertions of 3, 34, and 5 bases, respectively. The right part of the figure qualitatively shows for each mutant the activity of the origin of minus-strand replication as determined in vivo by the double origin plasmid method (5) and in vitro by the synthesis of primer RNA (7) and the ability to induce the SOS response as measured by β-galactosidase induction upon infection of an *E. coli* strain carrying a *dinD*::Mud(Ap *lac*) fusion (7). The asterisk indicates the synthesis of RNA shorter than the normal primer. SOS induction was scored as positive or negative when β-galactosidase activity (14) per A_{660} unit of culture at 120 min after phage addition was between 100 and 170 U or between 0 and 50 U, respectively.

boiled for 5 min in a sodium dodecyl sulfate (SDS)-loading buffer (12). The samples were loaded on a discontinuous 13% polyacrylamide gel containing SDS (12), and Western blotting and immunochemical detection were carried out as previously described (16, 17) with anti-LexA antibody and anti-immunoglobulin G coupled to horseradish peroxidase. The intensity of the LexA band was quantified by scanning the nitrocellulose membrane. Measurements were carried

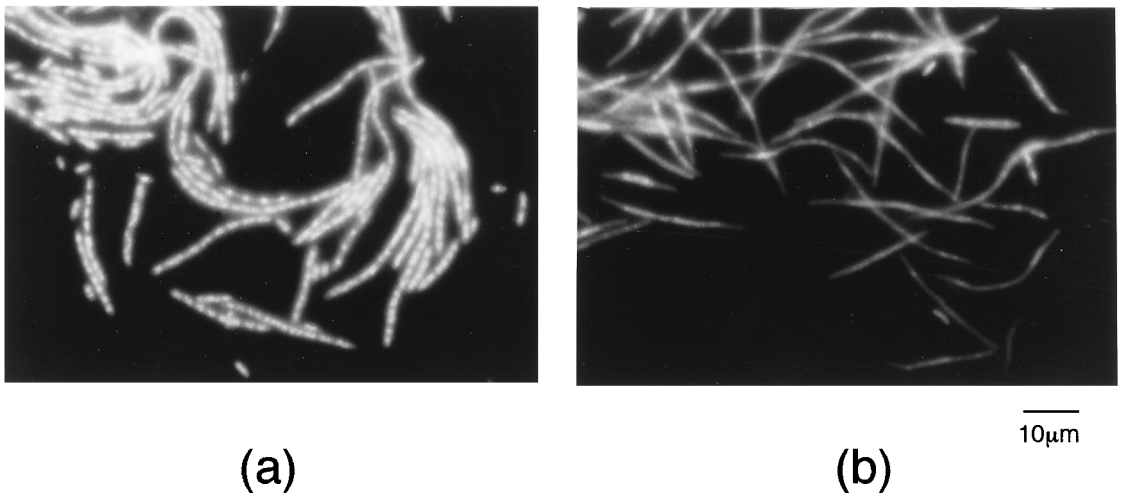

FIG. 3. Micrographs of *E. coli* AB1899 (*lon*) cells stained with DAPI (9) 2 h after infection with R377 (a) or treatment with mitomycin (0.25 μg/ml) (b).

out only within the range where the band intensity was proportional to the amount of the protein. The results shown in Fig. 1 indicate that infection with the mutant phage R377 reduced the cellular level of LexA in 5 to 10 min. Infection with the wild-type phage affected the level of LexA very little (Fig. 1). In the presence of this concentration of chloramphenicol, ssDNA of the wild-type phage was immediately converted to the double-stranded replicative form upon infection, but neither further replication of phage DNA nor protein synthesis was observed (data not shown). These results unambiguously show that the parental ssDNA of R377 by itself induced the SOS response in the infected cells.

From the data shown in Fig. 1, it appears as if the rate of LexA degradation in R377-infected cells is much lower than that in UV-irradiated cells. This lower rate probably does not reflect actuality and can be interpreted simply to reflect the slow and inefficient process of filamentous phage infection (13). Under the experimental conditions used, numbers of infective centers at 2, 5, 10, and 15 min after phage addition were 10, 40, 65, and 80%, respectively, of the total number of cells (data not shown). This result suggests that the total amount of LexA remaining in the culture at each time point after the addition of R377 (Fig. 1B) is essentially due to the number of uninfected cells. Under the same conditions, the amount of intracellular single-stranded phage DNA at 10 min after the addition of R377 was about 3 molecules per cell, which corresponds to 4.5 molecules per infected cell. No ssDNA (less than 0.3 molecule per cell) was detectable at 10 min after the addition of wild-type f1 (data not shown; see reference 7).

Correlation between SOS induction and origin function. The mutant phages R377 and M13ΔE101 (11), which induced the SOS response in the previous study (7), each carry a large deletion covering the entire origin of the minus-strand synthesis. We have isolated more mutants with smaller deletions or insertions and measured their origin activity (6) by both the double-origin plasmid assay in vivo (5) and the primer RNA synthesis assay in vitro (7). We then tested these mutants for their ability to induce the SOS response by measuring β-galactosidase production upon infection of JH137, a *dinD*::Mud(Ap *lac*) fusion strain (4, 7). The results summarized in Fig. 2 clearly show that the SOS inducibility is strictly correlated to defectiveness of the origin function. This, together with the observation that the SOS inducibility of M13ΔE101 was lost by inserting the minus-strand origin of phage G4 into its genome (7), indicates that the inducibility of the mutant phages is directly related to the loss of the origin function, not to the loss of other hypothetical functions possibly residing in the relevant region of the genome.

Normal replication of cellular DNA. Infection with the mutant filamentous phage provides a gentle method of inducing the SOS response. If SOS induction by mutant phage infection is simply due to the intracellular presence of parental ssDNA, cellular DNA replication is expected to continue normally, unlike in cases of mitomycin treatment or UV irradiation, even though cell division should be inhibited by the induced *sfiA* gene product. Figure 3 shows 4′,6-diamidino-2-phenylindole (DAPI)-stained AB1899 (*lon*) (10) cells that were either infected with R377 or treated with mitomycin. Although the cells formed filaments in both cases, it is clear that replication and partition of chromosomal DNA proceed correctly in R377-infected cells but not in mitomycin-treated cells.

We thank T. Horii and T. Ogawa of Osaka University and K. Tamai of the Medical Biological Laboratories for providing us with purified LexA protein and anti-LexA antibody, respectively.

This work was supported in part by grants-in-aid from the Ministry of Education, Science and Culture of Japan.

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