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SOS Induction by Stabilized Topoisomerase IA Cleavage Complex Occurs via the RecBCD Pathway▽†

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Accumulation of mutant topoisomerase I cleavage complex can lead to SOS induction and cell death in *Escherichia coli*. The single-stranded break associated with mutant topoisomerase I cleavage complex is converted to double-stranded break, which then is processed by the RecBCD pathway, followed by association of RecA with the single-stranded DNA.

DNA topoisomerases catalyze the interconversion of different DNA topological forms by coupling DNA strand passage with concerted breaking and rejoining of DNA (5, 43). *Escherichia coli* DNA topoisomerase I encoded by the *topA* gene plays an important role in the homeostatic regulation of DNA supercoiling (40, 41). It has an essential function in *E. coli* for preventing hypernegative supercoiling and R-loop formation (4, 19). Mutants lacking *topA* function fail to grow at low temperature (20, 37) and readily acquire compensatory mutations (10, 11, 30, 33). Mutants with a *topA* deletion and compensatory mutations have been shown to be defective in stress response because of their compromised ability to transcribe stress response genes (9, 13, 31, 32, 35, 39, 42). Loss of *topA* function also results in decreased resistance to extreme low pH (36).

DNA topoisomerases constitute an important class of therapeutic targets of anticancer and antibacterial agents (2, 27, 29). The drugs targeting topoisomerases achieve killing of cancer or bacterial cells not because the drugs inhibit the biological function of topoisomerases but because the drugs stabilize or increase the level of the covalent intermediate formed by topoisomerases with cleaved DNA during the catalytic cycle. Fluoroquinolones are highly potent antibacterial compounds that stabilize the covalent intermediates of DNA gyrase and topoisomerase IV (3, 12, 17). There is at least one type IA topoisomerase found in every bacterium examined thus far that is likely to be required for resolving entanglement of single strands of DNA during replication or recombination (43). Bacterial topoisomerase I could potentially be a useful target for development of novel antibacterial compounds to alleviate the need of new therapeutic drugs. However, since type IA topoisomerase cleaves a single-strand of DNA at a time, it was not clear whether the accumulation of such a cleavage complex would result in lethality for the bacterial cell. The potential of type IA topoisomerases as bactericidal targets was validated when a mutant of *Yersinia pestis* topoisomerase I, YTOP128, was isolated and characterized (6, 8). This mutant enzyme could cleave DNA and form the covalent complex but failed to religate the cleaved DNA due to a G122S substitution in the TOPRIM domain found among the three mutations identified on the topoisomerase coding sequence of YTOP128. The purified YTOP-G122S enzyme was also active in DNA cleavage but had no religation or relaxation activity (8). Overexpression of either the original YTOP128 mutant or the YTOP-G122S single substitution mutant topoisomerase I in *E. coli* led to a rapid loss of cell viability (8), with a slightly higher rate of cell killing for YTOP128 (6). These observations support the previous hypothesis that stabilization of covalent complex formed by bacterial type IA topoisomerase can lead to bacterial cell killing (14, 25). The *Y. pestis* topoisomerase I mutant YTOP128 was isolated originally by its ability to induce the SOS response of *E. coli*. Events that occur after the formation of the stable topoisomerase I cleavage complex leading to SOS induction, as well as the pathway of cell killing, remain unclear. Such information would shed light on how DNA damage from covalent protein-DNA complexes are repaired in *E. coli* and would also be useful for future evaluation of potential antibacterial compounds targeting topoisomerase I. The study reported here utilized the TOPRIM topoisomerase I mutant model system of YTOP128 and YTOP-G122S to examine the cellular response to stabilized topoisomerase I complex in *E. coli*.

Induction of SOS response by expression of YTOP128 and YTOP-G122S. The *dinD1*::*luxCDABE* on plasmid pDinlux (7) was utilized as a reporter of SOS induction. Expression of YTOP128 and YTOP-G122S from the BAD promoter on pAYTOP128 and pAYTOP-G122S (7) in *E. coli* strain BW27784 (15) with increasing concentrations of arabinose resulted in increase of luciferase signal (Fig. 1). There was no significant SOS induction from the expression of wild-type YTOP. The luciferase signal reached a maximum value at ca. 0.00006 to 0.0002% arabinose for both YTOP128 and YTOP-G122S. A further increase in arabinose concentration added to the culture resulted in drop of luciferase signal. This is probably due to the loss of cell viability resulting from the accumulation of high levels of topoisomerase I cleavage complex, since DNA religation is inhibited as a result of the G122S mutation (8). The presence of the M326V mutation in YTOP128 enhances DNA cleavage, with a 10- to 40-fold higher rate of cell killing (6) and also a higher luciferase activity from SOS induction (Fig. 1).

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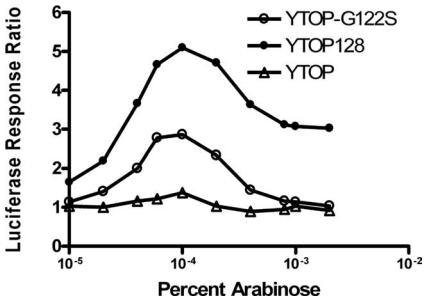

FIG. 1. Luciferase signal as reporter of SOS induction by mutant topoisomerase I cleavage complex. BW27784 transformed with luciferase reporter plasmid pDinlux along with either plasmid pAYOP (△), plasmid pAYTOP128 (●), or plasmid pAYTOP-G122S (○) was grown to exponential phase in LB medium with ampicillin and chloramphenicol at 37°C until the A_{600} reached 0.4. The culture was dispensed in 50-μl aliquots into white 96-well microtiter plates. Equal volume of LB medium containing 0.00001 to 0.004% arabinose was added for induction of recombinant *Y. pestis* topoisomerase I. The light production from the induced luciferase was measured on a Perkin-Elmer 7000 Plus BioAssay reader at 37°C in 10 min cycles with 30 s of shaking duration before each measurement. The luciferase response ratio was measured as the ratio of luciferase signal from the treated cultures versus the same culture that has not been treated with arabinose at 260 min after the addition of arabinose.

Effect of *recA*-null mutation on SOS induction and cell killing by topoisomerase I cleavage complex. We examined the effect of a complete loss of *recA* function on the cellular response to accumulated topoisomerase I cleavage complex. The constructions of the mutant derivatives of BW27784 are listed in Table S1 in the supplemental material. As shown in Fig. 2A, the luciferase signal from the *dinD1* promoter activity upon arabinose induction of YTOP-G122S was abolished in strain YT101 (*recA*::Tn*5*). The loss of SOS induction in YT101 was accompanied by a several-hundredfold decrease in cell viability measured 2 h after the addition of arabinose compared to the BW27784 parent (Table 1). These results from strain YT101 demonstrated not only that RecA function is involved in SOS induction after the accumulation of the topoisomerase I cleavage complex but also that RecA plays a role in protecting the bacterial cell from the killing effect of the topoisomerase I cleavage complex accumulation.

SOS induction is retained in the *recA718* mutant strain, but cell survival is compromised. To separate the SOS induction ability of RecA protein from its other functions in DNA repair, the *recA718* mutation was introduced into BW27784. Like wild-type *recA*, this allele requires DNA damage to become activated for SOS induction (21, 23). Luciferase measurement from *dinD1*::*luxCDABE* fusion after induction of YTOP-G122S with arabinose confirmed that strain JHS1 carrying the *recA718* allele responded to the topoisomerase I-mediated DNA damage with SOS induction (Fig. 2A). The luciferase response ratio was found to be slightly higher after the induction of both YTOP-G122S and YTOP128 in JHS1 than in BW27784 for arabinose concentrations between 0.00001 and 0.002% (Fig. 2B). Compared to *recA*$^+$ strains, strains with *recA718* mutation showed moderate UV sensitivity (45, 46), and the *recA718* mutation was lethal when combined with certain *polA* mutations (44) due to the effect of the *recA718* mutation on homologous recombination. It was hypothesized

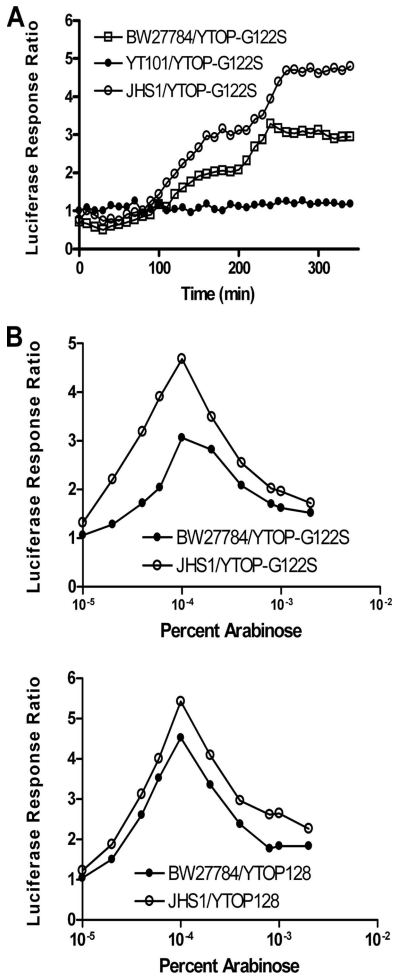

FIG. 2. Comparison of induction of *dinD1*::*luxCDABE* in strains with different *recA* genotypes after treatment with arabinose for the expression of recombinant mutant *Y. pestis* topoisomerase I. (A) The luciferase response ratio was measured in BW27784 (*recA*$^+$), YT101 (*recA*::Tn*5*), or JHS1 (*recA718*) as the ratio of luciferase signal from *dinD1*::*luxCDABE* in cultures induced with 0.0001% arabinose for the expression of YTOP-G122S versus luciferase signal from the same culture not treated with arabinose. (B) Luciferase response signal measured at 260 min after the addition of different concentrations of arabinose in BW27784 or JHS1 transformed with pAYTOP-G122S or pAYTOP128.

that RecA718 may perform certain recombinational repair processes less efficiently than wild-type RecA (44). JHS1 also showed a greatly decreased survival rate after the induction of YTOP-G122S compared to BW27784 (Table 1). These results from JHS1 showed that RecA homologous recombination function is directly involved in the repair of the topoisomerase I-mediated DNA lesion and that the SOS induction function of RecA is by itself insufficient for evading lethality due to the topoisomerase I-mediated DNA cleavage.

Extensive chromosomal fragmentation from the topoisomerase I-mediated DNA lesion. Chromosomal fragmentation occurs in *E. coli* in many situations when DNA lesions are formed and when *recA* is inactivated by mutation (16). If chromosomal fragmentation is increased significantly after trapping of the topoisomerase I cleavage complex, it would account for the

TABLE 1. Effect of *recA* mutations on relative cell viability following induction of wild-type or mutant *Y. pestis* topoisomerase I

Strain	Avg relative viability ± SD[a]			
	pAYTOP with:		pAYTOP-G122S with:	
	0.00006% arabinose	0.0002% arabinose	0.00006% arabinose	0.0002% arabinose
BW27784	0.87 ± 0.30	0.45 ± 0.20	0.065 ± 0.007	0.020 ± 0.003
YT101	1.05 ± 0.15	0.52 ± 0.19	$3.9 \times 10^{-4} \pm 1.5 \times 10^{-4}$	$6.0 \times 10^{-5} \pm 3.3 \times 10^{-5}$
JHS1	0.71 ± 0.40	0.19 ± 0.07	$9.2 \times 10^{-3} \pm 5.3 \times 10^{-3}$	$1.1 \times 10^{-4} \pm 3.4 \times 10^{-5}$

[a] The relative viability was measured as the ratio of viable counts determined 2 h after the addition of 0.00006 or 0.0002% arabinose versus the viable counts from control cultures not treated with arabinose. The results represent the average from three experiments.

higher rate of cell killing in YT101 when mutant topoisomerase I was induced. Pulsed-field gel electrophoresis was carried out under conditions such that the intact chromosomes stay in the wells, while the linear chromosomes enter the gel and the chromosomal fragments form a smear (16, 24, 38). None of the DNA entered the gel after induction of wild-type YTOP in BW27784 for 30 min with 0.0002 and 0.002% arabinose (Fig. 3, lanes 2 to 3). Linear chromosome and fragmented DNA can be observed after treatment with 0.5 mg of ciprofloxacin/liter for 30 min (lane 4). When mutant YTOP128 was induced in BW27784 with 0.00002 to 0.002% arabinose (lanes 6 to 9), chromosomal fragmentation could be observed after 30 min of incubation. There is some level of background DNA fragmentation in YT101 due to the *recA*-null mutation even with no arabinose added (lanes 10). When wild-type YTOP was induced in YT101 (lanes 11 to 14), additional chromosomal fragmentation over the background level could be observed, suggesting that, with the RecA function missing, overexpression of wild-type recombinant *Y. pestis* topoisomerase I could also result in increased DNA breaks from the large number of topoisomerase I cleavage events in the bacterial DNA genome. However, overexpression of wild-type recombinant *Y. pestis* topoisomerase I in BW27784 with the *recA*$^+$ genotype did not produce any DNA breaks (lanes 2 to 3). The chromosomal fragmentation in YT101 after the induction of YTOP128 by arabinose (lanes 16 to 19) could be readily observed as a smear of DNA entering the gel in addition to the linear chromosomal DNA, indicating the extensive accumulation of DNA breaks resulting from the cleavage complex formed by the mutant topoisomerase I. Western blot analysis of the total cellular proteins at 30 min after addition of arabinose showed that the expression levels of recombinant topoisomerase I with increasing arabinose concentrations were similar for wild-type YTOP and YTOP128 (see Fig. S1 in the supplemental material).

Requirement of RecBCD instead of the RecFOR complex for SOS induction and DNA repair after the accumulation of topoisomerase I-mediated DNA lesions. In *E. coli*, the RecA homologous recombination can be initiated after DNA damage via either the RecBCD pathway or the RecFOR pathway (34). The RecBCD complex is involved in processing of double-stranded DNA breaks before RecA catalyzed repair can take place (1). The RecFOR complex is mainly involved in single-strand gap repair in *E. coli* (34). After the treatment of *E. coli* with drug compounds that trap the covalent cleavage

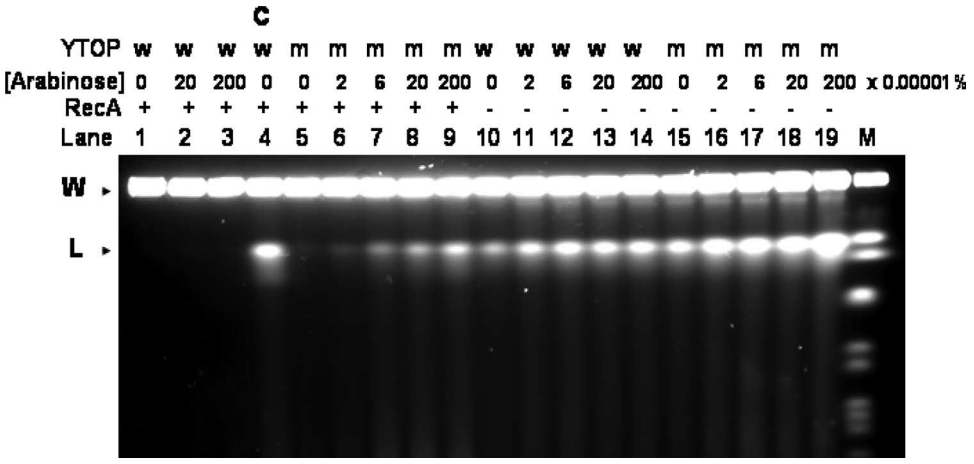

FIG. 3. Chromosomal fragmentation measured by pulsed-field gel electrophoresis. Exponential-phase cultures were induced with arabinose for the expression of wild-type and mutant recombinant *Y. pestis* topoisomerase I. After incubation with shaking at 37°C for 30 min, cells were collected by centrifugation and processed with the Bio-Rad CHEF bacterial genomic DNA plug kit. The agarose plugs containing the genomic DNA were electrophoresed in a 1% agarose gel with 0.5× Tris-borate-EDTA buffer with the Bio-Rad CHEF-DRII pulsed-field electrophoresis system (14°C, 6 V/cm, 21 h, linear ramping from 50 to 92 s), followed by staining with ethidium bromide. Analysis was carried out for BW27784 (lanes 1 to 9) or YT101 (*recA*::Tn*5*, lanes 10 to 19) cells transformed with pAYTOP (w, lanes 1 to 4 and lanes 10 to 14) or pAYTOP128 (m, lanes 5 to 9 and lanes 15 to 19). Chromosomal DNA gel plugs were prepared after 30 min of no treatment (lanes 1, 10, and 15), treatment with 0.5 mg ciprofloxacin/liter (C, lane 4), or arabinose at the indicated concentrations. W, gel wells; L, linear chromosome; M, *Saccharomyces cerevisiae* chromosome standards (225 to 2,200 kb).

TABLE 2. Effect of *recB*, *recF*, and *recR* mutations on luciferase response ratio from SOS induction by mutant topoisomerase I cleavage complex

Strain	Luciferase response ratio[a]	
	YTOP128	YTOP-G122S
BW27784	4.7	3.5
YT102 (*recB*)	0.91	0.96
JHS2 (*recF*)	4.1	3.4
JHS3 (*recR*)	4.4	3.9

[a] Luciferase response ratio was measured as the ratio of luminescence measured at 260 min after the addition of 0.00006% arabinose versus the luminescence from control cultures not treated with arabinose.

complexes formed by DNA gyrase, RecBC functions are required for SOS induction (26). However, DNA gyrase is a type IIA topoisomerase that cleaves a double strand of DNA at a time, while cleavage by the topoisomerase I would form a single-strand break on DNA. It is possible that repair of the topoisomerase I-mediated DNA break may involve the RecFOR gap repair pathway. We therefore measured the effects of *recB*, *recF*, and *recR* mutations in the BW27784 genetic background on SOS induction by the mutant YTOP cleavage complex. Luciferase measurement from *dinD1*::*luxCDABE* (Table 2) showed that SOS induction could not be observed in the presence of the *recB* mutation. The RecBCD pathway of double-stranded break repair thus appears to be required for generating the single-stranded DNA that becomes the substrate of RecA filament formation. In contrast, *recF* or *recR* mutation had little effect on SOS induction by the accumulated mutant topoisomerase I cleavage complex (Table 2), suggesting that the RecFOR gap repair pathways is not involved in the response. These conclusions from the SOS induction measurements were further supported by comparison of the viability of the *recB* mutant derivatives of BW27784 (YT102) versus the *recF* (JHS2) and *recR* (JHS3) mutants after induction of mutant YTOP-G122S (Fig. 4).

Similarity in cell killing pathway of type IA and type IIA topoisomerases. The formation of double-stranded breaks that are substrates of RecBCD is similar to the cell killing pathway of fluoroquinolone action (18, 26, 28). The conversion of the single-stranded break associated with topoisomerase I to a double-stranded break probably accounts for the rapid cell killing by the accumulated topoisomerase I-mediated DNA lesion due to the TOPRIM Gly-to-Ser topoisomerase I mutation (8).

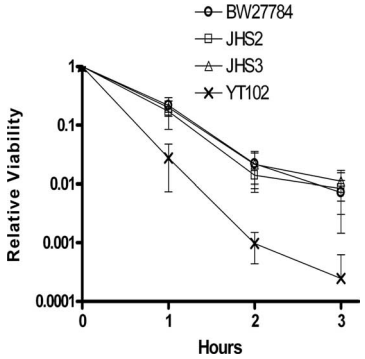

FIG. 4. Effect of *recB*, *recF*, or *recR* mutations on cell viability after induction of mutant *Y. pestis* topoisomerase I. Arabinose (0.002%) was added to the exponential-phase cultures of BW27784, YT102, JHS2, and JHS3 transformed with pAYTOP-G122S. Viable cell counts of arabinose-treated cultures determined after different periods of incubation at 37°C were divided by viable cell counts from untreated cultures to determine the relative viability. The results represent the averages and standard deviations from three experiments.

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