Mini-F plasmid-induced SOS signal in *Escherichia coli* is RecBC dependent

(F plasmid/mini-F *lynA* operon/indirect induction/RecBC nuclease activity)

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ABSTRACT Dispensable replicons such as F plasmid [95 kilobases (kb)] or its mini-derivatives such as mini-F (9.3 kb) or λ mini-F efficiently induced cellular SOS genes such as *sfia* (*sulA*) when they were damaged by UV irradiation and then introduced into a recipient bacterium. To generate an SOS signal, UV light-damaged mini-F or mini-F conditional mutants deficient in replication required that the bacterial RecBC enzyme retained some activity different from the nuclease activity that was dispensable. In contrast, UV light-damaged F plasmid produced an SOS signal independently of the activity of the RecBC enzyme and of the expression of the mini-F, -H, and -G proteins. Our findings are consistent with a picture in which the SOS signal is constituted by stretches of single-stranded DNA on a replicon. Moreover, our present data combined with other data previously published lead to the hypothesis that the SOS signal induced by mini-F plasmid is located *in trans* on the host chromosome, whereas the one generated by UV light-damaged F plasmid is *in cis* on the transferred DNA.

In *Escherichia coli*, when chromosome replication is affected by DNA lesions an SOS signal is produced that provokes the activation of RecA protein (for review, see ref. 1). Once activated, RecA protein promotes the cleavage of LexA and prophage repressors, resulting in the derepression of cellular SOS genes and prophages. The SOS pathway that follows the formation of an SOS signal is well documented (for review, see refs. 2–4).

In contrast, the pre-SOS pathway (that is, the process that generates an SOS signal) is not as well understood. Two main hypotheses have been proposed to account for the generation of an SOS signal. One states that the replication fork stops at a lesion and then resumes beyond the lesion, thus providing single-stranded DNA where RecA protein is activated (5). Another hypothesis is that repair involves DNA degradation by the *recBC* nuclease, thus producing oligonucleotides that activate RecA protein (6, 7).

To determine how an SOS signal is generated by DNA lesions, a few authors have resorted to using exogenous DNA damaged by UV irradiation and introduced into recipient cells (8–10). Then, it has been found that SOS induction is promoted by a class of closely related plasmids that seem to replicate and segregate in coordination with the bacterial chromosome. For example, an F plasmid [95 kilobases (kb)] carrying lesions induced by UV light efficiently induces prophage λ when introduced into a lysogenic host (11, 12), as does mini-F (9.3 kb), the quintessential replicon of F plasmid (13, 14).

Mini-F genes involved in the generation of an SOS signal have been identified (14). Furthermore, it has been demonstrated that mini-F can generate an SOS signal in the absence

of lesions produced by DNA-damaging agents. This so-called gratuitous induction is elicited when (i) mini-F carries a functional *lynA*⁺ locus, or (ii) mini-F cannot replicate or partition normally (15, 16). The *lynA* locus is an operon with two genes, *H* and *G*, that encode two small proteins (10.5 and 12 kDa) (17). If *H* protein fails to be synthesized as a consequence of an amber mutation in the *H* gene, then *G* protein has a lethal and SOS-inducing action (18). The two actions of *G* protein are prevented by *H* protein synthesized *in trans* by another plasmid (19, 20).

The current explanation for the SOS-inducing action of mini-F when it fails to partition or replicate normally is that the activity of *H* protein disappears, thus allowing *G* protein to generate an SOS signal (14–16, 19–21). Furthermore, we demonstrate here that the mini-F-induced SOS signal occurs in the absence of the RecBC nuclease activity. We also show that a conjugally transferred F plasmid damaged by UV light triggers a pre-SOS pathway different from the mini-F-induced pathway in that it is independent of the expression of *H* and *G* proteins and of the RecBC enzyme.

MATERIALS AND METHODS

Bacteria, Plasmids, and Phagemids. All strains listed in Table 1 are derivatives of *Escherichia coli* K-12. Two kinds of *recBC* mutants are used here: *recBC*[−] (null) mutants deficient in the ATP-dependent double-stranded DNA exonuclease and DNA unwinding activities, and *recBC*⁺ deficient in the ATP-dependent double-stranded DNA exonuclease activity (24, 25). Mini-F plasmids pML31*ts9* and pML31*ts17* are deficient in replication at 42°C (26). At the restrictive temperature, the miniF*ts9* mutation prevents mini-F from initiating replication, whereas the miniF*ts17* mutation affects replication at a later stage (26). The two temperature-sensitive (*ts*) mutations are located within the *Kpn* I fragment (43.6–46.9 kb) outside the *lynA* region (16). Plasmid pGY223 is pBR325 in which the mini-F *lynA* locus (42.85–43.6 kb) (14) was cloned. We used three λ mini-F phagemids—that is, λ vectors carrying mini-F (i) λ pML31: Kan^R (16), (ii) λ pSC138: Amp^R (27), and (iii) λ pSC138 *Ham22* (18). The latter fails to produce *H* protein in a *sup*⁺ host (17, 18).

Cultures and Media. Bacteria were grown in LBT (28) at 37°C or, when they carried a mini-F *ts* plasmid, in LBT supplemented with 25 μ g of kanamycin per ml at 28°C. Cell growth was monitored at OD₆₅₀ with a Varian 635 spectrophotometer. Synthetic media YM9C and YM9M were as described (29) except that uracil was omitted.

Direct and Indirect *sfia* Induction. We measured SOS induction by the induction of transcription of the *sfia* (*sulA*) gene. *sfia* induction was monitored by the amount of β -galactosidase produced in lysogens by a *sfia*::*lacZ*⁺ fusion carried by a λ c*lind1* prophage. Concentrations of β -galactosidase were calculated as described (30). Induction by

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Abbreviations: kb, kilobase(s); *ts*, temperature sensitive.
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Table 1. Bacterial strains

Strain	RecBC phenotype	Chromosomal marker					F	Source or origin; alias
		<i>recBC</i>	<i>recA</i>	<i>thyA</i>	<i>sfiA</i>	<i>lac</i>		
CSH55	+	+	+	+	+	Δ	—	Miller (22); GY2755
GC4473	+	+	441	+	85	<i>Y1</i>	—	Huisman and D'Ari (23); GY4337
GC4581	+	+	+	+	+	Δ	—	Huisman and D'Ari; GY4786
K2074	+	+	+	+	+	—	F <i>lac::Tn5</i>	Friedman via Reyes; GY4324
NK6600	—	B21	+	+	+	+	—	Kleckner via C. W. Roberts; carries <i>Tn10</i> in <i>argA</i> ; GY4335
V68	—	C73	+	+	+	+	—	Chaudhury and Smith (24); GY4343
V218	‡	B1009	+	+	+	+	—	Chaudhury and Smith (25); GY4344
V219	‡	C1010	+	+	+	+	—	Chaudhury and Smith (25); GY4345
GY6817	+	+	+	+	+	Δ	F <i>lac::Tn5</i>	CSH55 $\times$ K2074
GY6867	+	+	+	—	+	Δ	—	ThyA [−] mutant of GC4581
GY6888	‡	B21	+	+	+	Δ	—	Tet ^R <i>recB21</i> transduced into GC4581
GY6889	+	+	+	+	+	Δ	—	Tet ^R <i>recB⁺</i> transduced into GC4581
GY6920	—	B21	441	+	85	<i>Y1</i>	—	Tet ^R <i>recB21</i> transduced into GC4473
GY6924	—	B21	+	—	+	Δ	—	Tet ^R <i>recB21</i> transduced into GY6867
GY6925	+	+	+	—	+	Δ	—	Tet ^R transduced into GY6867
GY6930	‡	B1009	+	+	+	Δ	—	ThyA ⁺ <i>recB1009</i> transduced into GY6867
GY6931	‡	C1010	+	+	+	Δ	—	ThyA ⁺ <i>recC1010</i> transduced into GY6867
GY6932	—	C73	+	+	+	Δ	—	ThyA ⁺ <i>recC73</i> transduced into GY6867
GY6933	+	+	+	+	+	Δ	—	ThyA ⁺ transduced into GY6867

The strain Gif number is given as an alias. Strains GC4473, GC4581, and GY6867–GY6933 carry a noninducible prophage λ clind1 *sfiA::lacZ*⁺, in which *lacZ*⁺ is fused to the *sfiA* promoter (23). Phenotypes of *recBC* mutants are designated as minus when deficient in both nuclease and unwinding activities, and with a double dagger when deficient in nuclease activity and proficient in recombination and repair. (25). Mutation *recB21* was cotransduced by phage P1 with *Tn10* (Tet^R) inserted in *argA* from NK6600 into GC4473, GC4581, and its *thyA*⁺ derivative GY6867, which were sensitive to UV light and formed small colonies. The *recC73*, *recB1009*, and *recC1010* alleles from V68, V218, or V219 were cotransduced with *thyA*⁺ into GY6867 by phage P1. Loss of exonuclease activity was scored by the purple color of Thy⁺ transductants after replica plating onto EMBO plates overlaid with $\approx 10^7$ phage T4 *2amN51* (25).

thymine deprivation or expression of *recA441* (Tif) mutation was measured after incubation for 2 hr (23, 31). Indirect SOS induction was triggered (i) by the arrest of replication of mini-F *ts17* or mini-F *ts9* plasmids by temperature shift, (ii) by infection of recipient cells with UV light-damaged λ mini-F or λ mini-F *Ham22*, and (iii) by UV light-damaged F plasmid introduced by conjugation into recipient cells.

RESULTS

Two Independent Pre-SOS Pathways Are Triggered by the Arrest of Replication of Mini-F Plasmid and by UV Light Damage to the Cell Chromosome. Fig. 1 shows the time course of induction of *sfiA* triggered by UV irradiation and/or by the arrest of replication of mini-F plasmid by temperature shift.

FIG. 1. Kinetics of *sfiA* induction after arrest of mini-F *ts* replication. Samples of GC4581 bacteria with (squares) or without (circles) mini-F *ts9* at 10^8 per ml were diluted 1:10 into LBT at 42°C (open symbols) or exposed to UV light (5 J/m^2) before the temperature shift (closed symbols). *sfiA* induction was estimated by assaying β -galactosidase after various times of incubation at 42°C.

Damage by UV light-induced *sfiA* readily, and induction decayed rapidly after having reached a maximum. In contrast, after shifting mini-F *ts* carrier cells to 42°C, *sfiA* induction started after a lag of >1 hr and persisted for as long as 4 hr. When mini-F *ts* carrier cells were irradiated with UV light and shifted to 42°C, the curve of *sfiA* induction was biphasic: (i) the first wave mimicked the immediate but transient induction observed after UV irradiation of cells devoid of mini-F, and (ii) the second wave mimicked the induction curve of *sfiA* after the arrest of mini-F replication. Such a pattern showing the additivity of the two curves supports the independence of the two generated pre-SOS pathways.

The Pre-SOS Pathway Triggered by Arrest of Mini-F Replication Does Not Depend on the Nuclease Activity of the RecBC Enzyme. Gudas and Pardee (32) found that induction of RecA protein by nalidixic acid is inhibited in *recBC* null mutants. Similarly, *sfiA* induction after arrest of mini-F replication was inhibited in *recB21* and in *recC73* null mutants (Fig. 2). Note that *sfiA* was efficiently induced by UV light, or by Tif activation of *recA441* bacteria, or by thymine deprivation of Thy[−] cells, regardless of whether the cells were *recB21*. The induction ratio over the background by Tif activation was 58 in *recB⁺* and 94 in *recB21*; it was 20 in *recB⁺* and 29 in *recB21* after thymine deprivation.

Recently, new *recBC*‡ mutants have been isolated that have no detectable ATP-dependent double-stranded DNA exonuclease activity but retain recombination proficiency (25). Using such mutants, we demonstrated that the RecBC nuclease activity was not required for *sfiA* induction by arrest of replication of mini-F *ts* because *sfiA* was induced in *recB1009* or *recC1010* hosts as efficiently as in the *recBC*⁺ parent (Fig. 3).

Mini-F Deficient in H Protein Kills *recBC* Null Mutants but does Not Induce *sfiA*. Mini-F has both a lethal and an SOS-inducing action when deficient in the production of H protein (18). We observed that λ mini-F *Ham22* failed to

FIG. 2. Absence of *sfiA* induction by arrest of mini-F *ts* replication in *recBC* null mutants. GY6888: *recB21* (Δ , $\blacktriangle$), GY6932: *recC73* (∇ , $\blacktriangledown$), and GY6889: *recB*⁺ ($\square$, $\blacksquare$) bacteria carrying mini-F *ts17* were treated as described in Fig. 1. Irradiated and unirradiated bacteria are represented by closed and open symbols, respectively.

induce *sfiA* in a λ immune *sup*⁺ *recB21* recipient (Fig. 4). Host-cell killing after λ mini-F *Ham22* infection was the same in cells proficient or deficient in the RecBC enzyme; killing increased with increasing phagemid multiplicities (Fig. 5). Cells surviving λ mini-F *Ham22* infection were cured of the phagemid, whereas all the cells infected with wild-type λ mini-F survived and carried the wild-type phagemid (data not shown). Cell killing was also observed at 42°C in *recB21* and *recC73* bacteria carrying a resident mini-F *ts* plasmid (data not shown).

***sfiA* Induction by UV Light-Damaged F Plasmid is Independent of the Expression of H and G Proteins and Host RecBC Enzyme.** Since UV light-damaged F plasmid is transferred to a recipient as single-stranded DNA (33, 34) whereas UV light-damaged λ mini-F injects double-stranded DNA into the recipient cell, we questioned whether these two different DNAs would entail two different mechanisms of formation of the SOS signal. We found that the F plasmid introduced into an F⁻ recipient induced *sfiA* with rapid kinetics (Fig. 6 A and C), whereas λ mini-F caused induction only after a lag of >1 hr (Fig. 6 B and C). In recipients carrying a *lynA*⁺ plasmid that express H and G proteins, the transferred F plasmid efficiently induced *sfiA* (Fig. 6 A and C), whereas λ mini-F did not (Fig. 6 B and C). Moreover, F plasmid, but not λ mini-F, induced *sfiA* in a *recB21* recipient (Tables 2 and 3).

FIG. 3. Induction of *sfiA* by arrest of mini-F *ts* replication in *recBC*⁺ hosts. The *recBC*⁺ mutants GY6930 (mini-F *ts17*) (Δ) and GY6931 (mini-F *ts17*) (∇), the *recBC* null mutant GY6932 (mini-F *ts17*) ($\square$), and the wild-type GY6933 (mini-F *ts17*) ($\circ$) were diluted 1:10 in prewarmed LBT, incubated at 42°C to stop replication of mini-F *ts*, and periodically assayed for production of β -galactosidase. The kinetics of *sfiA* induction by mini-F *ts9* or mini-F *ts17* were similar (data not shown).

FIG. 4. Absence of *sfiA* induction by mini-F deficient in H protein synthesis in a *recB21* host. GY6888: *recB21* (triangles) and GY6889: *recB*⁺ (squares) were infected at a multiplicity of infection of 3, with (closed symbols) or without (open symbols) λ mini-F *Ham22*. After a 20-min adsorption period, the infected cells were diluted in LBT (time 0) and periodically assayed for production of β -galactosidase.

DISCUSSION

In this paper, we sought to determine how a replicon other than the bacterial chromosome produces an SOS signal when its replication is disturbed. F plasmid and its derivative mini-F were the two replicons tested.

When replication was impaired by UV light-induced lesions, we found that to produce an SOS signal, mini-F requires the RecBC enzyme and the expression of mini-F G protein, whereas F does not. Moreover, *sfiA* induction by mini-F was delayed by a few generation times, whereas induction by F plasmid was immediate, as also observed after cell exposure to UV irradiation.

The different structure of F and mini-F DNAs introduced into the host may account for their diverse kinetics and requirements for generating an SOS signal. A UV light-damaged F plasmid transmitted to a recipient consists of one DNA strand carrying lesions, with the other being synthesized during transfer. The latter strand is discontinuous; gaps

FIG. 5. Lethal effect on *recB*⁻ hosts of mini-F deficient in H protein synthesis. GY6888: *recB21* (squares) and GY6889: *recB*⁺ (circles) bacteria were infected with λ mini-F (open symbols) or λ mini-F *Ham22* (closed symbols). The initial numbers of bacteria and of input phagemids are indicated on the ordinate and on the abscissa, respectively. After a 10-min adsorption period at 37°C, diluted samples were plated on LBT plates to score host survival.

FIG. 6. *sfiA* induction by sexduction with UV light-damaged plasmid F is not inhibited by a *lynA*⁺ plasmid. (A) GY6817: *F lac*⁻ donors (8×10^8) exposed to UV light at 0 (open symbols) or 80 J/m² (closed symbols) were mated with 3×10^8 GC4581 (pGY223: *lynA*⁺) (triangles) or with 3×10^8 GC4581 (pBR325: *lynA*⁻) (circles) recipients. After 30 min at 37°C, the mating mixtures were diluted 1:2 and streptomycin (200 µg/ml) was added to prevent further growth of donor bacteria (time 0). β -Galactosidase was assayed after incubation of the mixtures for the periods indicated on the abscissa. (B) GC4581 (pGY223: *lynA*⁺) host cells 10^8 (triangles) and 10^8 GC4581 (pBR325: *lynA*⁻) bacteria (squares) were infected with 3×10^8 λ mini-F exposed to UV light at 0 (open symbols) or 350 J/m² (closed symbols). After a 20-min absorption period, the infected cells were diluted 1:10 into LBT (time 0) and β -galactosidase was assayed at times indicated on the abscissa. (C) Induction ratios were calculated as described in Table 2 from data in A and B. Symbols indicate induction ratios for recipients GC4581 (pGY223: *lynA*⁺) (▲, ▼) and GC4581 (pBR325: *lynA*⁻) (●, ■) after mating with UV-irradiated donors or after infection with UV-irradiated λ mini-F.

are formed opposite each photoproduct (33, 34) (Fig. 7) without the intervention of the RecBC enzyme. In contrast, mini-F injected into a recipient with a λ vector consists of double-stranded DNA; it may require the unwinding activity of the RecBC enzyme for generating an SOS signal (Fig. 7).

Our data raise the question of the location of the mini-F-induced SOS signal. Is it in *cis*, on mini-F itself, or in *trans* on the host chromosome?

Three arguments support the idea that this SOS signal may be located on host DNA and not on mini-F DNA. Firstly, the kinetics suggest it. The long latent period of SOS induction after the arrest of mini-F replication (16, 26) is more likely to represent something happening to the replicating host DNA than to the mini-F itself. Secondly, the mini-F-induced SOS signal persists in the host even after the loss of the mini-F plasmid (16). Thirdly, mini-F is known to kill the host and to disrupt chromosome segregation (20, 21).

It has been postulated that lack of replication or partition of mini-F leads to H protein inactivation, thus allowing G protein to induce SOS functions (14–16, 19–21) (Fig. 7).

Table 2. Induction of *sfiA::lacZ*⁺ after infection with UV light-damaged λ mini-F

Recipient	Infection by	Induction of <i>sfiA</i>		Induction ratio
		β -Galactosidase, units/ml	β -Galactosidase, units/mg	
GY6888: <i>recB21</i>	No infection	16.1	33.7	1
	λ mini-F	15.8	33.0	1
	λ mini-F*	16.2	39.4	1.2
GY6889: <i>recB</i> ⁺	No infection	36.5	54.0	1
	λ mini-F	44.0	65.1	1.2
	λ mini-F*	298.0	468.5	8.7

Recipients were infected with λ mini-F exposed to 0 or 500 J of UV light per m² (indicated by an asterisk). After a 20-min adsorption period, the infected cells were diluted, incubated for 3 (*rec*⁺) or 4 hr (*recB21*) at 37°C, and assayed for β -galactosidase. Uninfected recipients were treated similarly (rows 1 and 4). The induction ratio indicates the ratio of β -galactosidase (units/mg) produced by infected cells and uninfected cells.

How and where does G protein act? We proposed that the *lynA*-encoded proteins are the building blocks of a cellular apparatus that ensures the cosegregation of chromosome and F plasmid (14, 35). The disappearance of H protein may impair the functions of a "mitotic" apparatus, a structure binding DNAs to the cell envelope (Fig. 7).

A. Chaudhury and G. R. Smith (personal communication) have shown that there is SOS induction in *recBC* mutants by nalidixic acid, a gyrase inhibitor (37), suggesting that the RecBC activity required for SOS induction might be the unwinding of double-stranded DNA. SOS induction by mini-F G protein displays the same requirements. In the absence of H protein, G protein may inhibit a gyrase-mediated chromosome decatenation (for review, see ref. 36). Inhibition of resolution of daughter chromosomes followed by DNA unwinding by the RecBC enzyme may generate long stretches of single-strand DNA (Fig. 7).

Our data *in vivo* and data obtained *in vitro* (5) support the

Table 3. Induction of *sfiA::lacZ*⁺ by sexduction of *F*⁻ recipients with UV light-damaged F plasmid

Recipient	Cross	Induction of <i>sfiA</i>	
		(β -galactosidase, units/ml)	Induction ratio
GY6888: <i>recB21</i>	<i>F lac</i> ⁻ × <i>F</i> ⁻	2.6	1
	<i>F lac</i> ^{-*} × <i>F</i> ⁻	33.5	12.9
	<i>F lac</i> ⁻ × <i>F</i> ^{-*}	30.3	11.7
	<i>F lac</i> ^{-*} × <i>F</i> ^{-*}	47.0	18.1
GY6889: <i>recB</i> ⁺	<i>F lac</i> ⁻ × <i>F</i> ⁻	10.2	1
	<i>F lac</i> ^{-*} × <i>F</i> ⁻	81.7	8.0
	<i>F lac</i> ⁻ × <i>F</i> ^{-*}	75.6	7.4
	<i>F lac</i> ^{-*} × <i>F</i> ^{-*}	105.7	10.4

Donor bacteria GY6817: *F lac*⁻ exposed to 0 or 80 J of UV light per m² were mated with *F*⁻ recipients exposed to 0 or 10 J of UV light per m². UV-irradiated cells are designated by an asterisk. After 30 min of incubation at 37°C, tetracycline (10 µg/ml) was added to the mating mixtures to prevent further growth of donor bacteria. β -Galactosidase was assayed after a 90-min additional incubation. The induction ratio indicates the ratio of β -galactosidase (units/ml) produced in each cross by recipients over that produced in the cross of unirradiated recipients with unirradiated donors.

FIG. 7. Direct and indirect pre-SOS pathways.

hypothesis that disruption of replication or partition of a replicon leads to the formation of long-lasting single-stranded DNA constituting an SOS signal that activates RecA protein.

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