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Novel Mechanism of Cell Division Inhibition Associated with the SOS Response in *Escherichia coli*

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Certain *Escherichia coli* strains were shown to possess a novel system of cell division inhibition, called the SfiC$^+$ phenotype. SfiC$^+$ filamentation had a number of properties similar to those of *sfiA*-dependent division inhibition previously described: (i) both are associated with the SOS response induced by expression of the *recA*(Tif) mutation, (ii) both are associated with cell death, (iii) both are amplified in mutants lacking the Lon protease, and (iv) both are suppressed by *sfiB* mutations. SfiC$^+$ filamentation and *sfiA*-dependent division inhibition differed in (i) the physiological conditions under which loss of viability is observed, (ii) the extent of amplification in *lon* mutants, (iii) their genetic regulation (SfiC$^+$ filamentation is not under direct negative control of the LexA repressor), and (iv) their genetic determinants (SfiC$^+$ filamentation depends on a locus, *sfiC*$^+$, near 28 min on the *E. coli* map and distinct from *sfiA*).

Interruptions or perturbations of DNA replication in *Escherichia coli* cause a rapid cessation of cell division, resulting in filamentous growth (6). One mechanism ensuring this type of replication-division coupling has been analyzed in some detail. It involves the *sfiA* gene product, believed to be an inhibitor of cell division. The *sfiA* gene is repressed by the LexA repressor (17; S. Mizusawa, D. Court, and S. Gottesman, personal communication). After perturbations of DNA replication, the RecA protein acquires a protease activity and cleaves the LexA repressor, derepressing *sfiA* and a number of other operons called collectively the SOS functions (27). The high level of SfiA protein accumulated in the absence of LexA repressor results in a rapid arrest of cell division, probably by interacting with the *sfiB* gene product (28). When normal DNA replication is restored, the RecA protein loses its protease activity, LexA repressor accumulates, and the *sfiA* gene is again repressed. The SfiA protein accumulated during the induction period is rapidly destroyed in the presence of the Lon protease (31), permitting the resumption of cell division.

E. coli has been shown to possess a second, *sfiA*-independent replication-division coupling mechanism (2, 22), although its molecular bases have not been elucidated. The present work characterizes a third coupling mechanism, present in certain *E. coli* strains.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used are described in Table 1. The *recA441*(Tif) mutation was previously called *tif-1* (20). The *sfiA99*::Mu d(Ap *lac*) insertion is the *sfiA*::*lac* fusion described previously (17). Strain GC4540, obtained after infection with λ *c*I857 *b221 red* Tn*5*, contains a single, *pyrD*-linked Tn*5* sequence inserted in the *sfiA* gene and conferring a Sfi$^-$(Tr) phenotype. A Pro$^+$ SfiC$^-$ Ura$^-$ Km^r Str^r recombinant obtained from the cross GC2472 × GC2467 was transduced to Ura$^+$ (donor *pyrD*$^+$ *sfiA*$^+$) to obtain GC2480 (Km^s Ts) and GC2481 (Km^r Tr). A Km^r SfiC$^+$ Ura$^-$ Str^r recombinant from the cross GC2472 × GC380 was transduced to Ura$^+$ (donor *pyrD*$^+$ *sfiA*$^+$) to obtain GC2487 (Km^r) and GC2488 (Km^s); a Km^r SfiC$^-$ Ura$^-$ Str^r recombinant from the same cross was transduced to Ura$^+$ (donor *pyrD*$^+$ *sfiA*$^+$) to obtain GC2476 (Km^s Ts) and GC2477 (Km^r Tr).

The phage used were P1 *vir* for transduction, λ *rev der*$^-$ to test the *rac* allele (see below), and λ *c*I857 *b221 red* Tn*5* as Tn*5* donor, generously provided by O. Reyes and A. Toussaint.

Media. Rich medium was LB broth (30). Minimal 63 medium (30) was supplemented with glucose (0.4%) and, as needed, amino acids (100 μg/ml), Casamino Acids (CAA, 0.4%), uracil (20 μg/ml), and adenine or guanosine plus cytidine (100 μg/ml). Solid medium contained 1.5% Difco agar. Plates for transductions contained 5 × 10^{-3} M sodium citrate. The antibiotics used were streptomycin (200 μg/ml), kanamycin (25 μg/ml), and tetracycline (6 μg/ml).

Counting solution for the Coulter Counter was devised by F. Kepes. It contained 350 g of NaCl and 100 ml of Formol in 20 liters of distilled water. It was filtered twice through 0.45-μm Millipore filters before use.

Scoring of the SfiC phenotype. Cultures of *recA*(Tif) *sfiA* strains to be tested were grown into exponential phase at 30°C in 63 glucose–CAA medium (supplemented with tryptophan and uracil when needed). Adenine was added, and the cultures were incubated 3

TABLE 1. Bacterial strains

Strain	Relevant genotype				Source, reference, or construction
	recA	*sfiA*	*sfiC*	Other markers	
AB1157	+	+	(+)	*thr leu pro his arg lac gal rpsL rac*	15
GC2220	+	+	(+)	= AB1157 (P2)	Oscar Reyes
GC4413	441	99	+	*thr leu pyrD trp*::Mu *his* Δ*lac gal malB rpsL*	18
GC4415	+	99	+	= GC4413 *recA*$^+$ *srlC300*::Tn*10*	Tc^r *recA*$^+$ transductant, donor JC10236 (7)
GC2465	441	85	+	= GC4413 *pyrD*$^+$ *sfiA85*	Ura$^+$ Ap^s transductant, donor GC3218
GC4423	441	99	+	= GC4413 *leu*$^+$ *sfiB114*	18
JM12	441	+	+	= AB1157 *recA441*(Tif)	4
GC2467	441	100	+	= JM12 *sfiA100*::Tn*5*	Km^r Ura$^-$ transductant of JM12 (donor GC4540) transduced to Ura$^+$ (Km^r)
GC2480	441	+	1	*thr his arg rpsL*	See text
GC2481	441	100	1	*thr his arg rpsL*	See text
GC380	441	+	+	= AB1157 *recA441*(Tif) *lon-1 relA*	13
GC3218	441	85	2	*thr leu pro his arg gal rpsL relA*	13
GC2488	441	+	+	*thr leu pro his arg lac gal rpsL relA lon*	See text
GC2487	441	100	+	*thr leu pro his arg lac gal rpsL relA lon*	See text
GC2476	441	+	1	*thr leu pro his arg lac gal rpsL relA lon*	See text
GC2477	441	100	1	*thr leu pro his arg lac gal rpsL relA lon*	See text
GC2472	441	100	1	Hfr KL16 (PO 60 CCW) *pyrD prd relA*	Transduction of JM888 (5) to *prd*, then to Km^r Tr Ura$^-$ (donor GC4540)
LN1130	+	+	?	*leu thy trp*::Mu/F'123 *zcj*::Tn*10* Δ*rac*	J.-M. Louarn
GC4540	441	100	3	*thr leu his pyrD rpsL lexA*(Ts)	Km^r Tr derivative of GC4258 (20); see text

h at 42°C and then observed in the microscope. A uniformly filamentous population was scored SfiC$^+$, and uniformly small cells were scored SfiC$^-$. Occasionally, mixed populations were observed, including long filaments, small cells, and cells of intermediate length; these strains were scored SfiC$^+$.

Scoring of the *rac* allele. The phage λ *rev*, in which part of the *rac* cryptic prophage has replaced analogous λ genes, is able to grow on P2 lysogens; the mutant λ *rev der* has lost this ability. When it is propagated on *rac*$^+$ strains, however, it can recover the *der*$^+$ function by recombination with *rac* (33). We scored the *rac* allele by plating λ *rev der* on the strain to be tested, picking a plaque, and plating it on the *rac*$^-$(P2) strain GC2220. The efficiency of plating was about 10^{-4} for *rac*+ strains and 10^{-6} for *rac* strains.

Determination of mean cell volume. Samples were diluted 100-fold into filtered counting solution and analyzed in a model ZB Coulter Counter equipped with a 50-μm orifice and a 50-μl manometer. At an attenuation setting of 1/8 and an aperture current of 1/4, cells were counted in successive windows of 10 U with a lower threshold setting of 10 to 90. Larger cells were analyzed at an attenuation setting of 1/2 in successive windows of 5 U with a lower threshold setting of 25 to 95 (or 45 for nonfilamenting cultures). From the resulting volume distribution the mean volume was calculated. To convert units to μm^3, the Coulter Counter was calibrated by F. Kepes with latex beads 1.15 and 2.01 μm in diameter (Coultronics).

Photomicrographs. Unfixed bacterial preparations on glass slides were photographed in a Zeiss photomicroscope III with Ilford Pan F 50 ASA film.

Other methods. Conjugation and transduction were carried out essentially by the method of Miller (30). Optical density was measured at 600 nm in a Gilford 240 spectrophotometer. UV irradiation was with a Sylvania germicidal lamp at an intensity of 1 W/m^2.

RESULTS

The SfiC$^+$ phenotype. The *sfiA* and *sfiB* mutants were selected as thermoresistant, UV-resistant survivors of the *recA*(Tif) *lon* strain GC380 (13). The *recA*(Tif) mutation alters the RecA protein in such a way that it acquires protease activity and induces the SOS response spontaneously at 42°C in the presence of adenine, without any externally applied perturbation of DNA replication (4). The *lon* mutation inactivates the Lon protease, permitting greater accumulation of SfiA protein (31). Thus, the *recA*(Tif) *lon* parental strain filaments and loses viability rapidly at 42°C in the presence of adenine. The *sfiA* and *sfiB* derivatives, on the other hand, show normal cell division under these conditions (13).

In the course of our work on the *sfiA*-dependent mechanism of division inhibition, we discovered that strain GC4413 *recA*(Tif) *sfiA99*::Mu d(Ap *lac*) filamented in liquid culture at 42°C in the presence of adenine (Fig. 1A), although the efficiency of colony formation was 100% at 42°C on plates of the same composition.

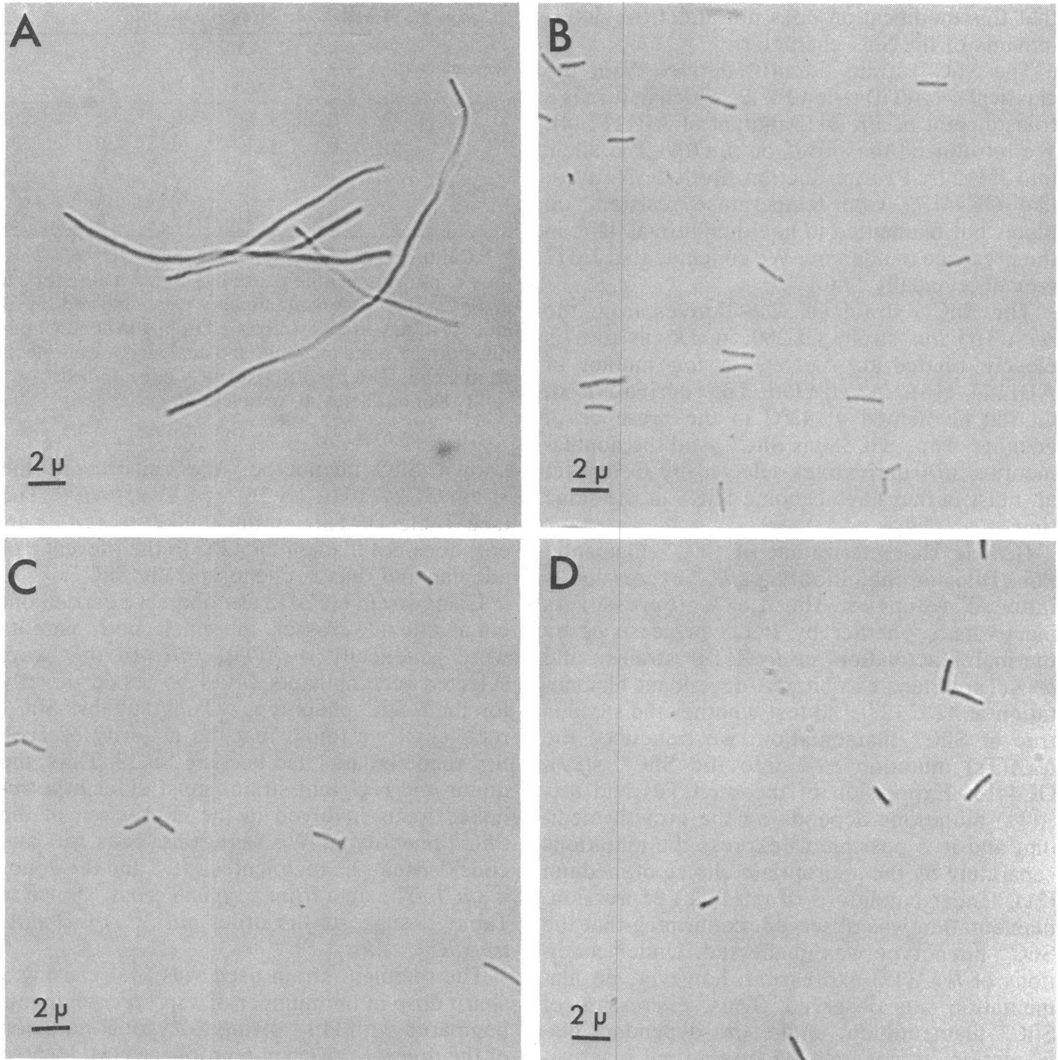

FIG. 1. Cultures growing exponentially at 30°C in glucose-CAA medium (plus uracil and tryptophan for A, B, and C) were supplemented with adenine and incubated 2 h at 42°C. Bacteria were mounted on slides, and photomicrographs were taken as described in the text. Strains were as follows: (A) GC4413 *recA*(Tif) *sfiA*; (B) GC4415 *sfiA*; (C) GC4423 *recA*(Tif) *sfiA sfiB*; (D) GC2481 *recA*(Tif) *sfiA sfiC*.

The filamentation was clearly due to expression of the *recA*(Tif) mutation since it was not observed at 42°C in the presence of guanosine and cytidine, conditions known to suppress Tif-mediated induction of λ, filamentation, mutagenesis, and synthesis of RecA (3, 26, 36). Furthermore, no filamentation was observed in a *recA*$^+$ derivative of GC4413 at 42°C in the presence of adenine (Fig. 1B). These results indicate that this division inhibition is part of the SOS response.

The nature of the *sfiA* allele in GC4413 did not affect the phenotype of the strain. Introduction by P1 transduction of the *sfiA85* allele did not abolish filamentation at 42°C in the presence of adenine. In control experiments, the original *recA*(Tif) *sfiA85* strain GC3218 showed normal cell division at 42°C in the presence of adenine.

We shall call Tif-mediated *sfiA*-independent filamentation the SfiC$^+$ phenotype. It follows from this definition that the SfiC phenotype can only be tested in a *recA*(Tif) *sfiA* genetic background.

The SfiC$^+$ and SfiC$^-$ strains GC4413 and GC3218 are both closely related to the widely used K-12 strain AB1157. To trace the origin of their divergence, we tested the SfiC phenotype of the earliest *recA*(Tif) ancestor of each strain. To do this, we first had to introduce a *sfiA* mutation into the two strains. We show below

that this modification does not affect the determinants of the SfiC character.

The SfiC$^+$ strain GC4413 derives from the classical *recA*(Tif) strain JM12, which in turn is a transductant of a *recA13* mutant of AB1157 (4). We introduced the *sfiA85* or *sfiA100*::Tn*5* allele into JM12 by P1 transduction. Both derivatives, like GC4413, were temperature resistant on plates but filamented in liquid culture at 42°C in the presence of adenine. We conclude that JM12 is phenotypically SfiC$^+$.

The SfiC$^-$ strain GC3218 derives from the *recA*(Tif) *lon* strain GC380, which in turn is closely related to AB1899, a *lon* mutant of AB1157 (13). A *sfiA100*::Tn*5* derivative of GC380 filamented at 42°C in the presence of adenine. Thus, GC380 is SfiC$^+$, and the nonfilamenting *sfiA* derivatives selected by George et al. must in fact have become SfiC$^-$ at the same time.

Genetic characterization of SfiC$^+$ filamentation. Division inhibition via *sfiA*$^+$ expression is induced whenever the LexA repressor is inactivated, whether by RecA protease or by thermal inactivation in *lexA*(Ts) strains, and *lexA*(Ts) strains exhibit *sfiA*-dependent filamentation at 42°C (29). To test whether the same is true of SfiC$^+$ filamentation, we tranduced the *lexA*(Ts) mutation *tsl-1* into the SfiC$^+$ strain GC4413. Expression of the *recA*(Tif) and *lexA*(Ts) mutations depends on the growth medium, and it is possible to express the mutations separately by the appropriate choice of medium (21). Under conditions of *recA*(Tif) expression, filamentation was observed, confirming that the SfiC$^+$ phenotype was unaffected. Under conditions of *lexA*(Ts) expression, however, no filamentation was observed. Thus, expression of SfiC$^+$ filamentation, unlike *sfiA*-dependent filamentation, is not induced by thermal inactivation of LexA repressor and must require RecA protease activity for something other than (or in addition to) cleavage of LexA repressor.

The *sfiB* mutations do not affect induction of *sfiA* expression but make cells insensitive to the high levels of SfiA product synthesized (18). These mutations, recently shown to lie in the *ftsZ* gene (28), probably modify the target on which the SfiA protein normally acts, making it insensitive to inhibition by SfiA (28). To test whether SfiC$^+$ filamentation is similarly affected, we transduced the *sfiB114* mutation into the SfiC$^+$ strain GC4413. The resulting strain no longer filamented at 42°C in the presence of adenine (Fig. 1C). Similarly, a *leu*$^+$ *sfiB103* transductant of the SfiC$^+$ strain JM12 was SfiC$^-$ (data not shown). Thus, SfiC$^+$ filamentation is suppressed by *sfiB* mutations.

To identify the genetic determinants of the SfiC$^+$ phenotype, we needed an Hfr donor of known SfiC phenotype. We constructed the strain GC2472 Hfr KL16 *recA*(Tif) *sfiA100*::Tn*5* (see Table 1). This strain, unlike its *sfiA*$^+$ parent, does not filament at 42°C in the presence of adenine and thus is phenotypically SfiC$^-$.

TABLE 2. Mapping of *sfiC*[a]

Recombinant	Alleles[b]				No.
	pyrD	*sfiA*	*trp*	*sfiC*	
1	0	0	1	0	10
2	0	1	1	0	3
3	1	1	1	0	1
4	0	0	1	1	13
5	0	1	1	1	5

[a] Cultures of strains GC2472 and GC2465 were grown into exponential phase in LB and mated for 2 h at 30°C. Trp$^+$ Str^r recombinants were selected on 63 glucose–CAA–uracil–streptomycin plates at 30°C, purified on the same medium, and analyzed for unselected markers. The *pyrD* marker was injected last.

[b] 1, Donor alleles; 0, recipient alleles.

Using strain GC2472 as donor, we carried out conjugational crosses in which both parents were genetically *recA*(Tif) *sfiA*. In this way, selected recombinants could be tested directly for their SfiC phenotype. Using suitable SfiC$^-$ recipients, we found that the majority of Trp$^+$ Str^r recombinants had become SfiC$^-$. Thus, the donor and recipient strains must differ in a *trp*-linked locus involved in the expression of the SfiC$^+$ phenotype. We name this locus *sfiC* and consider that the recipient is *sfiC*$^+$ and the donor is *sfiC1*. The data from a typical cross, shown in Table 2, suggests the order *gal . . . pyrD-sfiA-trp-sfiC . . . his*.

The recipient strain used was *rac*, causing a sharp drop in the number of Trp$^+$ recombinants compared with His$^+$ owing to zygotic induction of the donor *rac*$^+$ cryptic prophage (11), located at 30 min (1). The fact that no *sfiC* clones were recovered among 40 selected His$^+$ recombinants suggests that *sfiC* is injected after *rac*. The SfiC phenotype is independent of *rac*, as shown by the isolation of all four combinations of *sfiC* and *rac* alleles.

In P1 transduction experiments with *sfiC1* strains as donors and the *sfiC*$^+$ strains GC4413 or GC2465 as recipients, no cotransduction was observed between *sfiC* and *pyrD* at 21 min (0 of 40 transductants), *trp* at 27 min (0 of 45), or *zcj*::Tn*10* at 29 min (0 of 38). The *sfiC* gene is thus clearly distinct from *sfiA*, which is about 50% cotransducible with *pyrD* (12, 13, 21, 25). All the mapping data together suggest that the *sfiC* locus is near 28 min or, less likely, between 22 and 26 min.

Physiological characterization of SfiC$^+$ filamentation. We compared the division arrest in *recA*(Tif) strains carrying different combinations

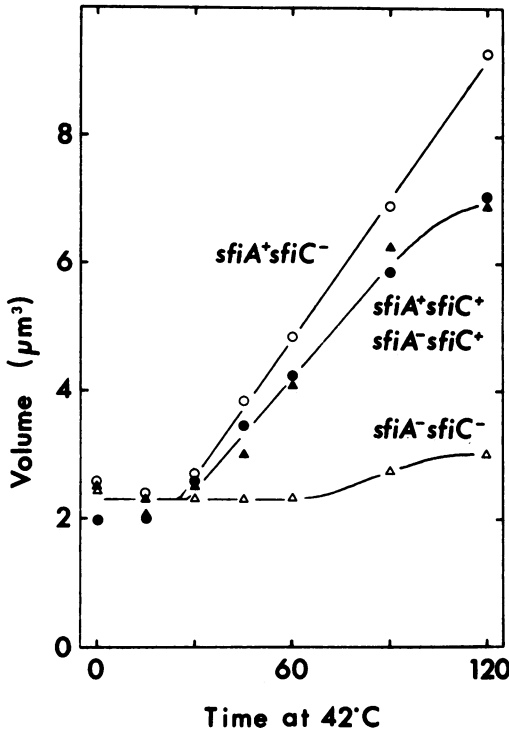

FIG. 2. Volume of cultures growing exponentially at 30°C in 63 glucose-CAA medium supplemented with adenine and shifted to 42°C at time zero. Samples were withdrawn periodically, and the average volume was determined with the aid of a Coulter Counter as described in the text. Symbols: ●, JM12 (*sfiA*$^+$ *sfiC*$^+$); ▲, GC2467 (*sfiA sfiC*$^+$); ○, GC2480 (*sfiA*$^+$ *sfiC*); △, GC2481 (*sfiA sfiC*). All strains carried the *recA*(Tif) mutation.

of *sfiA* and *sfiC* alleles. Exponentially growing cultures were shifted to 42°C, and adenine was added at time zero. At various times, the cell volume distribution was determined with a Coulter Counter, and the average cell volume was calculated. The results (Fig. 2) show that mean cell volume starts to increase about 25 min after the temperature shift in *sfiA*$^+$ and *sfiC*$^+$ cultures. Little increase in cell number was observed in these cultures. The precise amount of residual division is difficult to evaluate, however, since filaments are counted less efficiently than cells of normal size, leading to an apparent drop in cell concentration at late times. The *recA*(Tif) *sfiA sfiC* strain, on the other hand, showed no division inhibition: mean cell volume remained essentially constant (Fig. 2), and viable cell concentration increased 13-fold in 120 min. Microscopic observation confirmed that the cells were of normal size (Fig. 1D).

Since filamentation is often associated with cell death, we monitored survival during *recA*(Tif) expression. The *sfiC*$^+$ strains lost viability after 2 h at 42°C (Table 3). Expression of *sfiA*$^+$, on the other hand, did not cause cell death (in an *sfiC* strain) during 2 h in liquid medium (Table 3). On solid medium the situation was reversed, the *sfiA*$^+$ allele causing severe loss of viability during overnight incubation at 42°C and the *sfiC*$^+$ allele being neutral (Table 3).

In *lon* mutants, the SfiA protein is stabilized (31), making the cells hypersensitive to treatments that induce SfiA synthesis. To see whether a *lon* mutation similarly sensitizes cells to the presence of *sfiC*$^+$, we constructed a series of *recA*(Tif) *lon* strains carrying all combinations of *sfiA* and *sfiC* mutations and examined their viability under conditions of *recA*(Tif) expression. After 2 h of incubation at 42°C in the presence of adenine, both *sfiA*$^+$ and *sfiC*$^+$ strains showed loss of viability (Table 3). Compared with the *lon*$^+$ strains under these conditions, the *lon* mutants exhibited lethality which was 13-fold greater in the presence of the *sfiC*$^+$ allele and 20,000-fold greater in the presence of *sfiA*$^+$. On solid medium at 42°C, the *sfiC*$^+$ allele still exerted a slight lethal effect on the *lon* strains, whereas the *sfiA*$^+$ allele caused severe loss of viability (Table 3). Thus, the absence of the Lon protease sensitizes cells to the presence

TABLE 3. Lethal effect of *sfiC*$^+$ filamentation[a]

Genotype		*recA*(Tif)			*recA*(Tif) *lon*		
sfiA	*sfiC*	Strain	EOP (42°C)[b]	Survival (42°C)[c]	Strain	EOP (42°C)	Survival (42°C)
+	+	JM12	1.7×10^{-5}	0.11	GC2488	6×10^{-6}	6×10^{-5}
−	+	GC2467	1.1	0.12	GC2487	0.24	9.2×10^{-3}
+	−	GC2480	1.4×10^{-5}	1.2	GC2476	1.5×10^{-5}	5.5×10^{-5}
−	−	GC2481	1.0	9.8	GC2477	0.89	5.8

[a] Cultures growing exponentially in glucose-CAA medium at 30°C were assayed on LB plates at 30°C and on 63 glucose-CAA-adenine plates at 42°C; the ratio of the two titers is the efficiency of plating at 42°C. A portion of each culture was supplemented with adenine, incubated 2 h at 42°C, and again assayed on LB plates at 30°C; the ratio of this titer to that of the unheated culture is the survival after 2 h in liquid medium at 42°C.

[b] EOP, Efficiency of plating; see footnote *a*.

[c] See footnote *a*.

of both *sfiA*$^{+}$ and *sfiC*$^{+}$ alleles, although not to the same extent.

DISCUSSION

Expression of the *recA*(Tif) mutation in *E. coli* results in induction of the SOS response, including a rapid inhibition of cell division. This division inhibition has been reported to require the *sfiA* gene product (13), synthesis of which is induced massively whenever the LexA repressor is inactivated (17).

The present work shows that in certain *E. coli* strains expression of the *recA*(Tif) mutation can also cause *sfiA*-independent division inhibition. By analogy with *sfiA*, we have called the genetic element responsible for this filamentation the *sfiC* locus. Although other explanations cannot be ruled out, the data are consistent with the hypothesis that the *sfiC*$^{+}$ gene product, like *sfiA*$^{+}$, is synthesized at high levels during expression of the SOS response and results in division inhibition.

In several respects, *sfiA*- and *sfiC*-dependent division inhibitions are similar. Both are bona fide SOS functions, expressed in *recA*(Tif) strains at 42°C in the presence of adenine and leading to a rapid block of cell division. The *sfiB* mutations, which probably alter the target of action of SfiA (28), suppress both types of filamentation, suggesting that the *sfiA*- and *sfiC*-dependent mechanisms may block septation at the same step.

Both types of division inhibition are associated with cell death, and in both cases the lethal effect is amplified in *lon* mutants. For *sfiA*, this amplification has been shown to be due to the stabilization of the SfiA protein in *lon* strains (31), suggesting that SfiA may be a natural substrate of the Lon protease.

The *sfiC*$^{+}$ function, when present, mimics that of *sfiA*$^{+}$ in certain respects. Its presence could account for several apparent paradoxes. The original selection of the *sfi* mutants involved 4 h of *recA*(Tif) expression in liquid culture followed by overnight expression on plates at 42°C (13). Thus both *sfiA*- and *sfiC*-dependent lethality were counterselected, explaining the recovery of either *sfiA sfiC* double mutants or *sfiB* mutants in which both types of filamentation were suppressed. The frequency at which these mutants were found was 10^{-8}, much lower than the frequency of 10^{-4} to 10^{-6} at which *sfiA* mutants are found by selection on plates (12, 20, 35), conditions under which *sfiC*$^{+}$ is not lethal.

The strain WP44$_{s}$ of Witkin is a hybrid between the K-12 *recA*(Tif) strain JM12 and B/r (genotype *lon sfiA*). It carries the *recA*(Tif) mutation of JM12 (36) and the *sfiA* (or *sulA*) mutation of B/r (E. Witkin, personal communication). The strain filaments during expression of the *recA*(Tif) mutation (36). If this division inhibition is due to the *sfiC*$^{+}$ allele of JM12, it would explain why in constructing WP44$_{s}$ only 1 of 150 selected Thy^{+} recombinants filamented (E. Witkin, personal communication): although *recA* is closely linked to *thyA*, the *sfiC*$^{+}$ allele would have been injected much later.

The division inhibitions dependent on *sfiA* and *sfiC* differ with respect to the physiological conditions under which they cause loss of viability during expression of the *recA*(Tif) mutation. In liquid culture, 2 h at 42°C causes significant killing in *sfiC*$^{+}$ strains but not in *sfiA*$^{+}$ (*sfiC lon*$^{+}$) strains. This may reflect more rapid recovery from induction of *sfiA* through turn-off of *sfiA* expression (19) and degradation of the SfiA protein (31). Overnight incubation at 42°C on solid media, on the other hand, results in efficient killing by the *sfiA*$^{+}$ function, whereas *sfiC*$^{+}$ is essentially neutral.

The *sfiA* and *sfiC* loci are genetically distinct. Moreover, their regulation appears to be different. The *sfiA* gene is negatively controlled by the LexA repressor (17; S. Mizusawa, D. Court, and S. Gottesman, personal communication). The *sfiC* gene, on the other hand, is not directly under LexA control since a *lexA*(Ts) mutation does not cause *sfiC*-dependent filamentation at 42°C. In this respect, *sfiC* resembles a gene of an inducible cryptic prophage, repressed by the prophage repressor and induced by RecA protease. In fact, there is a formal analogy between the *sfiC* locus and the λ *kil* gene: derivatives of λ lysogens in which prophage replication, excision, and late functions are deleted have been shown to filament when the prophage repressor is inactivated owing to expression of the *kil* gene in the left arm of λ (14). This filamentation, however, unlike *sfiC*-dependent division inhibition, is not suppressed by the *sfiB114* mutation (F. Bernardi, personal communication).

The SOS response in some *E. coli* strains is thus seen to include several independent functions leading to division inhibition: *sfiA*$^{+}$, *sfiC*$^{+}$, *kil* (in λ lysogens), and possibly an additional function revealed in *ruv* mutants (32, 34). A number of other SOS functions (see reference 27) also have formal analogs among inducible prophage functions (see reference 9). The λ *c*I repressor, like LexA, is a substrate of the RecA protease and is an autorepressor. To the λ *kil*, *red*, and *int* functions, negatively regulated by *c*I (9), correspond the *sfiA*, *recA*, and *himA* functions, repressed by LexA (27). UV irradiation of *E. coli* induces an inhibition of DNA degradation, an alleviation of restriction of unmodified DNA, and a release of polarity (10, 27); these genetically unidentified SOS manifestations are reminiscent of the λ *gam*, *ral*, and N functions (8, 9, 37).

The SOS response is induced specifically when DNA replication is perturbed. Cell division, on the other hand, is tightly regulated, even during unperturbed growth. We have investigated possible roles of the *sfiA*$^+$ function in normal division regulation and have shown in particular that it is not involved in determining cell mass at different growth rates, mass adjustment after a nutritional shift-up, and chromosome segregation during steady-state growth (23). The strains used in these studies were genetically *sfiC* (unpublished data), so the processes studied must be *sfiC*-independent as well.

A *sfiA*-independent mechanism of cell division inhibition has been shown to come into play during thymine starvation (22) and after UV irradiation or nalidixic acid treatment (2). In all cases, filamentation started later than in *sfiA*$^+$ strains and was not suppressed by the *sfiB114* mutation. We have further shown that the *sfiA* strains involved in both studies were genetically *sfiC* (unpublished data). Therefore, *E. coli* must possess a division inhibition mechanism independent of both *sfiA* and *sfiC*. This is consistent with earlier reports of filamentation during thymine starvation in *recA* (24) and *lexA*(Ind$^-$) (16) mutant strains, in which the entire SOS response is uninducible.

Thus, the tight coupling of cell division to DNA replication observed in *E. coli* during thymine starvation or after UV irradiation is ensured on the one hand by the inducible SOS response, which includes the *sfiA*$^+$ and *sfiC*$^+$ division inhibition functions, and on the other hand by an additional mechanism, independent of the SOS response.

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