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Dissociation of *tsl-tif*-Induced Filamentation and *recA* Protein Synthesis in *Escherichia coli* K-12

OLIVIER HUISMAN,* RICHARD D'ARI, AND JACQUELINE GEORGE†

Institut de Recherche en Biologie Moléculaire, Centre National de la Recherche Scientifique, 75221 Paris Cedex 05, France

In *Escherichia coli*, expression of the *tif-1* mutation (in the *recA* gene) induces the "SOS response" at 40°C, including massive synthesis of the *recA*(*tif*) protein, cell filamentation, appearance of new repair and mutagenic activities, and prophage induction. Expression of the *tsl-1* mutation (in the *lexA* gene) induces massive synthesis of the *recA* protein and cell filamentation at 42°C, although other SOS functions are not induced. In this paper we show that the septation inhibition induced in *tif* and *tsl* strains at 42°C is not due to the presence of a high concentration of *recA* protein since (i) no *recA* mutants ($\leq 10^{-8}$) were isolated among thermoresistant nonfilamenting revertants of a *tif-1 tsl-1* strain, (ii) in a *tsl-1 zab-53* strain, only the low basal level of *recA* protein was synthesized at 42°C, yet cell division was inhibited, and (iii) in a *tsl-1 recA99* (amber) strain, no *recA* protein could be detected at 42°C, yet cell division was inhibited. Among suppressors of *tsl-tif*-induced lethality are mutations at a locus which we call *infB*, located in the 66- to 83-min region. The *infB1* mutation confers a highly pleiotropic phenotype, which is suggestive of a regulatory defect; it suppressed *tsl-tif*-induced filamentation but not *recA* protein synthesis, it did not suppress ultraviolet-induced filamentation (in a *lon* derivative), and it reduced but did not abolish *tif*-mediated induction of λ prophage and bacterial mutagenesis. The dissociation of *tsl-tif*-induced septation inhibition and *recA* protein synthesis in the *tif-1 tsl-1 infB1* strain suggests that the control of SOS filamentation may not be strictly identical to the control of *recA* protein synthesis.

In nonlysogenic *Escherichia coli* expression of the *tif-1* mutation at 40°C causes an inhibition of septation, resulting in the formation of long, filamentous cells (8, 16). The induction of filamentation under these conditions is one aspect of a highly pleiotropic cellular response, which is called the "SOS response" (31). It includes the induction of massive synthesis of the *recA* protein, the appearance of new DNA repair and mutagenic activities, and (in λ lysogens) prophage induction (40). The SOS response, which is thermally inducible in *tif-1* mutants, can be induced in *tif*$^+$ strains by unscheduled stops in DNA replication (e.g., after UV irradiation) (40).

The *recA* and *lexA* functions are necessary for induction of the SOS response, whether by *tif* expression or by a DNA replication block. The mutations *recA*, *zab*, *lexB* (all at the *recA* locus [10]), and *lexA* prevent induction of all aspects of the SOS response and render the bacteria hypersensitive to DNA-damaging treatments, such as UV irradiation (6, 9, 29). The temperature-sensitive "SOS constitutive" mutation *tif-1* (*recA441*) is also located in the *recA* gene (10, 14, 18, 23). No known *lexA* allele confers a Tif$^-$ phenotype, which causes induction of the entire SOS response at high temperatures. The very interesting *tsl* mutations, which have been isolated from UV-resistant pseudorevertants of *lexA* mutants and are probably located in the *lexA* gene (29), cause thermal induction of filamentation and of *recA* protein synthesis without a concomitant induction of λ prophage (17, 28). This partial induction, unlike all other known means of inducing the SOS response, does not depend on normal *recA* function, for it takes place in a *tsl-1 recA1* strain at 42°C (17, 30).

Satta and Pardee (34) have shown that under a broad range of conditions it is not possible to dissociate the filamentation response and *recA* product induction. To account for this striking similarity of induction patterns, they proposed that high concentrations of *recA* product may directly cause septation inhibition. In this paper we show that *tsl*-induced filamentation can occur in the absence of *recA* product. Furthermore, a mutation which we call *infB* suppresses *tsl-tif*-induced filamentation but not *recA* product synthesis; it confers a pleiotropic phenotype char-

† After 5 years of heroic struggle against cancer, Jacqueline George passed away on 14 August 1979. Despite weakened health and debilitating therapy, she continued to stimulate and participate in the work of the microbial genetics group which she created.

acteristic of regulatory mutations, suggesting that the regulation of the filamentation response may not be strictly identical to the regulation of *recA* product synthesis.

MATERIALS AND METHODS

Bacterial and phage strains. The bacterial strains used in this study are listed in Table 1. All are *E. coli* K-12; λ lysogenic derivatives were constructed as described previously (12). JM1 is the AB1157 strain of Howard-Flanders et al. (21), which is reported to carry in addition the markers *xyl*, *mtl*, *ara*, *thi*, *tsx*, and *supE*. All *tsl-1* strains presumably carry the suppressed *lexA3* mutation (29), and the *zab-53* strain GC4348 carries the suppressed *tif-1* mutation (9). The *tif-1* mutation has been shown to lie in the *recA* gene (10, 14, 18, 23) and should thus be assigned a *recA* allele number. We propose that it be called *recA441*. For ease of comprehension, however, we continue to employ the familiar designation *tif-1* below.

The phage used were λ *ref*, P1 *vir*[s] for transduction and φ80 *am2 supF* for strain construction involving the *recA99* amber mutation.

Media. LB medium contains (in grams per liter): yeast extract (Difco), 5; tryptone (Difco), 10; and NaCl, 10. Thymine (50 μg/ml) was added as needed. LB medium plates were solidified with 1.5% agar (Difco).

The minimal medium used was M63 (25) supplemented with glucose (0.4%), thiamine (10 μg/ml), and the required amino acids (100 μg/ml). Enriched minimal medium (EMM) was supplemented with Casamino Acids (0.4%); EMMA was EMM supplemented with adenine (100 μg/ml). Uracil (20 μg/ml) and streptomycin sulfate (200 μg/ml) were added as needed. Minimal medium, EMM, and EMMA plates were solidified with 1.5% agar (Difco). N medium (28) contains 8 g of Difco tryptone per liter, 5 g of NaCl per liter, 0.2% glucose, 0.25% Casamino Acids and 10 μg of thiamine per ml; NA medium was N medium supplemented with adenine (100 μg/ml). NA plates were solidified with 1.2% Difco agar.

Semienriched minimal medium was minimal medium supplemented with nutrient broth (800 μg/ml;

TABLE 1. *Bacterial strains*

Strain	Relevant marker(s)[a]	Other markers	Source, derivation, and/or reference
JM1		*thr leu his argE proA lac gal rpsL*	8
GC2004	*tif-1 malB*	*thr leu his proA lac gal rpsL*	12
GC4148	*tif-1 tsl-1*	*thr leu his proA lac gal rpsL*	*malB*$^+$ *tsl-1* transductant of GC2004; donor, DM962
GC4149	*tif-1*	*thr leu his proA lac gal rpsL*	*malB*$^+$ *tsl*$^+$ transductant of GC2004; donor, DM962
GC4158	*tsl-1*	*thr leu his proA lac gal rpsL*	*malB*$^+$ *tsl-1* transductant of GC2002 (12); donor, DM962
GC4191	*tif-1 tsl-1 infB1*	*thr leu his proA lac gal rpsL*	Thermoresistant revertant of GC4148
GC4189	*tif-1 tsl-1 infB9*	*thr leu his proA lac gal rpsL*	Thermoresistant revertant of GC4148
GC4258	*tif-1 tsl-1 pyrD34*	*thr leu his lac rpsL*	*pro*$^+$ *gal*$^+$ *pyrD34* recombinant of GC4247 × GC4148.
GC4259	*tif-1 tsl-1 sfiA11*	*thr leu his lac rpsL*	*pyrD*$^+$ *sfiA11* transductant of GC4258; donor, GC3001 (15)
GC4298	*tif-1 tsl-1 aroB*	*thr leu his pyrD34 lac rpsL*	*aroB* derivative of GC4258
GC4337	*tif-1 tsl-1 ilvA*	*thr leu his argE proA cycC lac gal rpsL*	*tif tsl-1 ilvA* derivative of JM1
GC4325	*tif-1 tsl-1 infB1 lon-1*	*thr leu his gal rpsL*	*lac*$^+$ *pro*$^+$ *lon-1 rpsL* recombinant of GC306 × GC4191
GC4336	*tif-1 tsl-1 lon-1*	*thr leu his gal rpsL*	*lac*$^+$ *pro*$^+$ *lon-1 rpsL* recombinant of GC306 × GC4148
GC4348	*tsl-1 zab-53*	*thr leu his proA lac gal rpsL*	*arg*$^+$ *tsl-1 rpsL* recombinant of DM962 × JM1253 (9)
GC4293	*tsl-1 recA99*	*rpsL*	*sup*$^+$ derivative of *gal*$^+$ *tsl*$^+$ *rpsL* recombinant of DM962 × DM456 (Φ80 *am2 supF*) (27)
DM962	*tsl-1*	Hfr Ra2 *rha* (PO 87 C W)	D. Mount
GC403		Hfr AB313 (PO 83 CCW) *ilv leu thyA lac str*	J. George
GC414		Hfr KL25 (PO 83 CW)	J. George
GC297		HfrG6 *his* (PO 66 CW)	J. George
GC306	*lon-1*	HfrH (PO 96 CW)	J. George
GC4247	*pyrD34*	HfrH *proC* (PO 96 CW)	J. George

[a] *tif-1* is now called *recA441* (see text).

Oxoid), threonine, leucine, arginine, guanosine, and cytidine (each at 100 μg/ml), and isoleucine and valine (each at 40 μg/ml). Semienriched minimal medium plates were solidified with 1.5% Difco agar.

Strain construction and genetic analysis. P1 *vir*-mediated transduction and bacterial crosses were carried out as described previously (8, 9).

Selection of temperature-resistant revertants of *tif-1 tsl-1*. A total of 10 independent cultures of strain GC4148 were grown in N medium to an optical density at 650 nm of 0.6. Samples (0.1 ml) were plated onto prewarmed NA medium plates and incubated overnight at 42°C; 10 clones from each culture were purified on NA medium plates at 42°C and tested for their UV sensitivity at 30 and 42°C at doses of 5, 10, 20, and 40 J/m^2.

Coulter Counter determination of cell concentration. Cells were counted with a model ZB ZBI Coulter Counter equipped with a 50-μm orifice and a 50-μl manometer. An attenuation setting of 1/2, an aperture current setting of 1/4, a lower threshold of 5, and an upper threshold of 100 were used for all counts. Samples (0.2 ml) were added to 25 ml of filtered Isoton II (Coultronics); cultures were appropriately diluted in growth medium in order to keep particle counts per 50 μl between 6,000 and 60,000.

Detection of the *recA* protein. Total bacterial protein was extracted from 5 ml of a culture at an optical density at 650 nm of 0.5. The technique of Gudas and Pardee (19) was used. The entire sample was layered onto a sodium dodecyl sulfate-polyacrylamide gel.

***tif* and UV mutagenesis.** For *tif* mutagenesis, exponential-phase cultures growing at 30°C in EMM at a concentration of 1×10^8 to 2×10^8 bacteria per ml were centrifuged, suspended in 10^{-2} M $MgSO_4$, and plated at 30 and 42°C onto histidine-limiting (0.8 μg/ml) minimal medium plates supplemented with adenine (100 μg/ml). For UV mutagenesis, exponential cultures were centrifuged, suspended in M63 buffer, UV irradiated or not treated, and plated in the dark at 30 and 42°C onto semienriched minimal medium plates. Prototrophic His$^+$ revertants were titrated by spreading 0.1-ml portions of undiluted cultures and incubating for 72 h. During this time, the growth-limiting amount of histidine was depleted, and only the His$^+$ revertants were able to form colonies. Viable bacteria were also assayed on histidine-limiting minimal medium or semienriched minimal medium plates; because of the small inoculum, the residual growth permitted each viable cell to form a visible colony.

RESULTS

***tsl*-induced filamentation is not due to high concentrations of *recA* protein. (i) Absence of *recA*-linked suppressors of *tsl*-induced filamentation.** The *recA1* missense mutation (18) does not suppress *tsl*-induced filamentation (30) or *recA* protein synthesis (17) in a *tsl-1 recA1* strain at 42°C, although it abolishes the induction of *recA* protein synthesis after nalidixic acid treatment (17). We wanted to determine whether by direct selection we could isolate mutations at the *recA* locus which, unlike *recA1*, suppress *tsl*-induced filamentation. To increase the selective pressure, we used a *tif-1 tsl-1* strain.

The *tif-1* and *tsl-1* mutations do not show the same medium dependence for their expression. Thus, to select temperature-resistant revertants, it was necessary to find a medium in which both mutations could be expressed. NA medium proved to be satisfactory (Table 2); indeed, the plating efficiency of the *tif-1 tsl-1* double mutant on NA medium plates at 42°C was less than the product of the plating efficiencies of the two single mutants.

Temperature-resistant revertants of the *tif-1 tsl-1* strain GC4148 were recovered on NA medium plates after one night at 42°C at frequencies of approximately 10^{-6}. Of 100 revertants purified and tested, only 1 proved to be UV sensitive. The mutation responsible for temperature resistance and UV sensitivity was shown by Hfr crosses to be in the *lexA* region. This was confirmed by transducing the mutation into the *tif-1 malB* strain GC2004; 50% of selected Mal$^+$ transductants were thermoresistant and UV sensitive, which is consistent with reported *malB-lexA* cotransduction frequencies (28). In all probability this revertant acquired an intragenic suppressor of *tsl-1*, unmasking the *lexA3* mutation presumed to be present in *tsl-1* strains (29). Thus, by direct selection for suppression of *tsl-tif*-induced lethality, no UV-sensitive *recA* mutants were isolated ($\leq 10^{-8}$).

Since the degree of UV sensitivity associated with different mutations at the *recA* locus is variable (10, 26), we analyzed 10 independent UV-resistant revertants. Hfr crosses indicated that at least two different loci were involved. In eight of the strains the suppressor was in the *sfiA* region. This was confirmed by transducing these suppressor mutations into the *tif-1 tsl-1 pyrD* strain GC4258; in all eight cases about 50% of selected Pyr$^+$ transductants were thermoresistant, which is consistent with the reported *pyrD-sfiA* cotransduction frequency (15).

The other two temperature-resistant revert-

TABLE 2. *Medium dependence of tif and tsl expression*[a]

Strain	Relevant genotype	Efficiency of colony formation at 42°C on:[b]		
		EMMA	LB medium	NA medium
GC4149	*tif-1*	2.4×10^{-4}	1.0	1.5
GC4158	*tsl-1*	1.0	2.1×10^{-3}	4.8×10^{-3}
GC4148	*tif-1 tsl-1*	1.4×10^{-4}	2.1×10^{-3}	2.5×10^{-6}

[a] Exponential-phase cultures of each strain growing at 30°C in EMM, LB broth, or N medium were assayed at 30 and 42°C on EMMA, LB medium, or NA medium plates, respectively.

[b] Ratio of titer at 42°C to titer at 30°C.

ants, GC4191 and GC4189, carried suppressors unlinked to the *recA*, *lexA*, *sfiA*, or *sfiB* locus; we designate these new suppressor mutations *infB1* and *infB2*, respectively. These two strains were crossed with different Hfr strains, and selected recombinants were tested for thermosensitivity. Table 3 indicates that the *infB*$^+$ allele was injected early by Hfr's G6 and AB313 but not by KL25 (Fig. 1), locating *infB1* and *infB2* between 66 and 83 min on the *E. coli* genetic map (3). No cotransduction was observed between *infB1* and *aroB* (73 min) or *ilvA* (83 min) among 250 Aro$^+$ or 240 Ilv$^+$ transductants, when the recipient strains GC4298 (*tif tsl aroB*) and GC4337 (*tif tsl ilvA*) were used. More precise mapping of the *infB* mutations is in progress.

Thus, no *recA*-linked suppressors of *tsl*-induced filamentation ($\leq 10^{-7}$) were found among UV-resistant heat-resistant revertants of the *tif-1 tsl-1* strain.

TABLE 3. *Mapping of the infB mutations*[a]

Donor strain	Recombinants selected	No. of temperature-sensitive recombinants[b]	
		infB1[c]	*infB2*[d]
Hfr G6 (GC297)	Thr$^+$ Leu$^+$ (Str^r)	16/28	12/25
Hfr 1B313 (GC403)	His$^+$ (Thy$^+$)	3/28	4/25
Hfr KL25 (GC414)	Thr$^+$ Leu$^+$ (Str^r)	0/28	0/25

[a] Standard crosses were carried out in LB broth at 30°C; after 90 min, mating mixtures were centrifuged and plated onto selective plates at 30°C.

[b] Recombinants purified on selective plates at 30°C were grown and tested for temperature sensitivity by streaking on NA medium plates at 30 and 42°C.

[c] Recipient strain, GC4191 (*tif-1 tsl-1 infB1*).

[d] Recipient strain, GC4189 (*tif-1 tsl-1 infB2*).

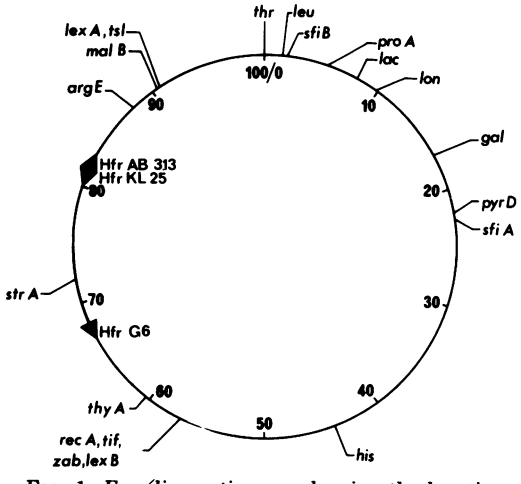

FIG. 1. *E. coli genetic map showing the locations of the principal markers used (3).*

(ii) *tsl*-induced filamentation in the absence of massive synthesis of the *recA* protein. Two mutations at the *recA* locus, *zab-53* and *recA99*, have been reported to suppress *tsl*-mediated induction of *recA* protein synthesis (23, 24). Our failure to isolate *recA*-linked suppressors of *tsl*-induced filamentation prompted us to investigate the cell division pattern in *tsl-1 zab-53* and *tsl-1 recA99* strains at 42°C.

The *zab-53* mutation, which was isolated as a suppressor of *tif* (9), has been shown by complementation studies to affect the *recA* locus (10). It also suppresses UV induction of λ prophage and confers a UV-sensitive phenotype (9). It differs from the *recA1* mutation in that it suppresses induction of the *recA* protein in a *tsl-1 zab-53* strain at 42°C, although the low constitutive level of this product is not noticeably affected (24; unpublished data). Nevertheless, cell division was still arrested in the *tsl-1 zab-53* strain at 42°C (Fig. 2). Furthermore, the lethality associated with expression of the *tsl-1* mutation was not affected; the colony-forming capacity of this strain on LB medium plates at 42°C was 8×10^{-4} relative to that at 30°C.

Not even the basal level of *recA* protein can be detected at 30 or 42°C in a *tsl-1 recA99 sup*$^+$ strain in which the *recA* gene bears an unsuppressed amber mutation (23; unpublished data). Nevertheless, cell division was completely arrested in this strain at 42°C (Fig. 2), and the colony-forming capacity on LB medium plates at 42°C was 3×10^{-4}.

Thus, *tsl*-induced lethal filamentation occurs in *zab-53* and *recA99* strains in the absence of high levels of *recA* protein.

Regulation of the filamentation response is not identical to the regulation of *recA* protein synthesis. The *sfiA11* and *sfiB114* mutations were isolated as specific suppressors of *tif*-induced filamentation (15). They also suppress *tsl*-induced filamentation (24; unpublished data) and, in *lon* derivatives, UV-induced filamentation (15). Other aspects of the SOS response are not affected; in particular, induction of massive synthesis of *recA* protein is not suppressed by these mutations (18, 39; unpublished data). The total and specific suppression of SOS filamentation by *sfiA* and *sfiB* mutations strongly suggests that these loci define elements directly involved in the septation inhibition and not in its regulation.

The *infB* mutations described above were also selected to suppress *tsl-tif*-induced filamentation. A more extensive characterization of the *tif-1 tsl-1 infB1* strain, however, revealed a

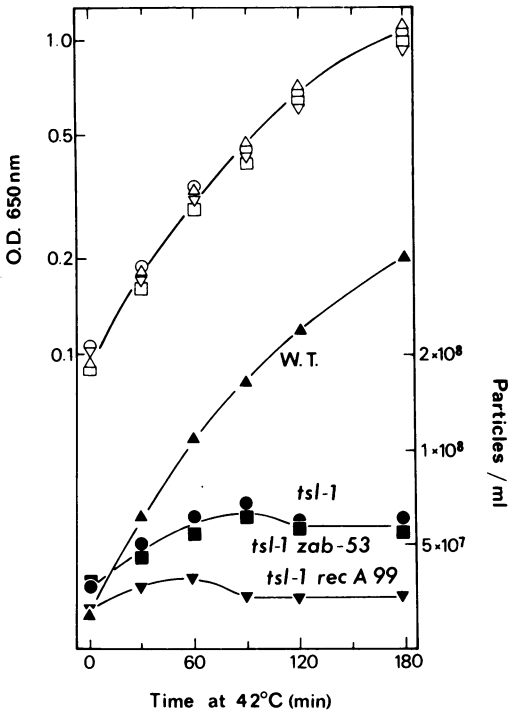

FIG. 2. *Inhibition of cell division in tsl-1 zab-53 and tsl-1 recA99 strains. Exponential-phase cultures growing in N medium at 30°C were diluted and shifted to 42°C at zero time, and samples were withdrawn periodically for measurements of optical density at 650 nm (O.D. 650 nm) (open symbols) and cell counts, as determined with a Coulter Counter (closed symbols). The strains used were JM1 (wild type)* (△ *and* ▲), *GC4158 (tsl-1)* (○ *and* ●), *GC4348 (tsl-1 zab-53)* (□ *and* ■), *and GC4293 (tsl-1 recA99)* (▽ *and* ▼).

highly pleiotropic phenotype, which was more suggestive of a regulatory defect. Since the filamentation response and *recA* product induction were affected in very different ways, we present the *infB1* phenotype below.

(i) Suppression by *infB1* of *tsl-tif*-induced filamentation but not of *recA* protein synthesis. We first determined to what degree the *infB1* mutation suppressed the division arrest induced by expression of the *tif-1* and *tsl-1* mutations. Cultures of *tif-1 tsl-1*, *tif-1 tsl-1 infB1*, and *tif-1 tsl-1 sfiA11* strains were shifted to 42°C in NA medium and incubated for several hours. Microscopic observations revealed uniformly long filaments in the *tif-1 tsl-1* population and no filaments in the *tif-1 tsl-1 infB1* and *tif-1 tsl-1 sfiA11* cultures. Quantitative measurements of cell mass and cell number under the same conditions showed (Fig. 3) that the complete arrest of cell division observed in the *tif-1 tsl-1* population within 30 min of the shift to 42°C was suppressed by the *infB1* and *sfiA11* mutations.

lon mutants are abnormally sensitive to SOS filamentation and lethality; low doses of UV irradiation cause irreversible, lethal filamentation (21), *tif-1 lon-1* strains lose viability at 40°C more rapidly and more completely than *tif-1 lon*$^+$ strains (15), and *tsl-1 lon-1* strains similarly show greater loss of viability at 42°C than *tsl-1 lon*$^+$ strains (unpublished data). In all cases the amplification of SOS lethality is suppressed by *sfiA* or *sfiB* mutations (15, 21a). We wanted to test whether the *infB1* mutation could suppress *tsl-tif*-induced filamentation, even as amplified by the presence of a *lon* mutation. A culture of the *tif-1 tsl-1 infB1 lon-1* strain GC4325 grown for several hours in NA medium at 42°C contained only the low proportion of filaments always found in *lon* cultures at all temperatures; a parallel culture of the nearly isogenic *infB*$^+$ strain GC4336 contained only long filaments. Similarly, the efficiency of colony formation of the *tif-1 tsl-1 infB1 lon-1* strain in NA medium plates at 42°C was 1, compared with 8×10^{-6} for the *tif-1 tsl-1 lon-1* strain.

We next investigated the effect of the *infB1* mutation on the induction of *recA* protein synthesis by expression of the *tif-1* and *tsl-1* mutations. In both the *tif-1 tsl-1* and *tif-1 tsl-1 infB1*

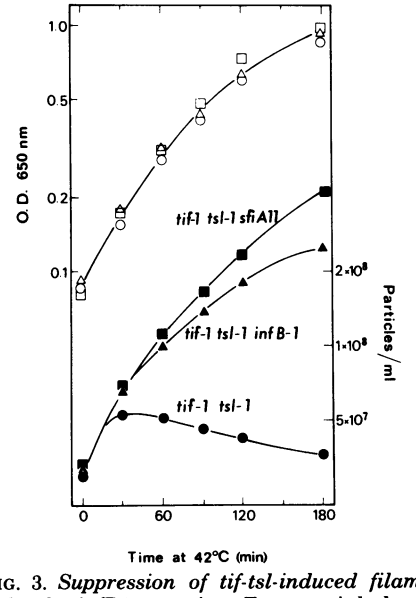

FIG. 3. *Suppression of tif-tsl-induced filamentation by the infB1 mutation. Exponential-phase cultures growing in N medium at 30°C were diluted into prewarmed NA medium at zero time and shifted to 42°C. Samples were withdrawn periodically for measurements of optical density at 650 nm (O.D. 650 nm) (open symbols) and cell counts (closed symbols). The strains used were GC4148 (tif-1 tsl-1)* (○ *and* ●), *GC4259 (tif-1 tsl-1 sfiA11)* (□ *and* ■), *and GC4191 (tif-1 tsl-1 infB1* (△ *and* ▲).

strains, the relative amount of *recA* protein detectable on sodium dodecyl sulfate-polyacrylamide gels was clearly increased after 2 h of incubation in NA medium at 42°C (Fig. 4), although densitometer tracings indicated that less *recA* protein was made in the *tif-1 tsl-1 infB1* strain in 2 h.

Thus, the *infB1* mutation completely suppresses filamentation and lethality in the *tif-1 tsl-1 infB1* strain at 42°C in NA medium but still permits clear induction of *recA* protein synthesis.

(ii) UV-induced filamentation in *infB1* bacteria. To determine whether the *infB1* mutation, like *sfiA* and *sfiB* mutations, totally suppresses all SOS filamentation, we examined its effect on the filamentation response after UV irradiation. For this, we again took advantage of the *lon-1* mutation, which sensitizes the cells to UV irradiation by amplifying the SOS-associated, *sfi*-dependent division inhibition. We compared the UV inactivation curves of *infB1 lon*$^+$ and *infB1 lon-1* strains by plating onto LB medium at 30 and 42°C; a clear sensitization was observed in the *lon-1* derivative, quantitatively identical to that observed in isogenic *infB*$^+$ strains (Fig. 5). Microscopic examination of *infB1 lon-1* and *infB*$^+$ *lon-1* cultures exposed to 10 J of UV irradiation per m^2 and then incubated for several hours in LB medium at 30°C revealed that the bacteria formed long filaments in both cultures. Thus, the *infB1* mutation, unlike *sfiA* and *sfiB* mutations, does not prevent UV-induced filamentation in *lon-1* strains and thus is not a total suppressor of SOS-associated filamentation.

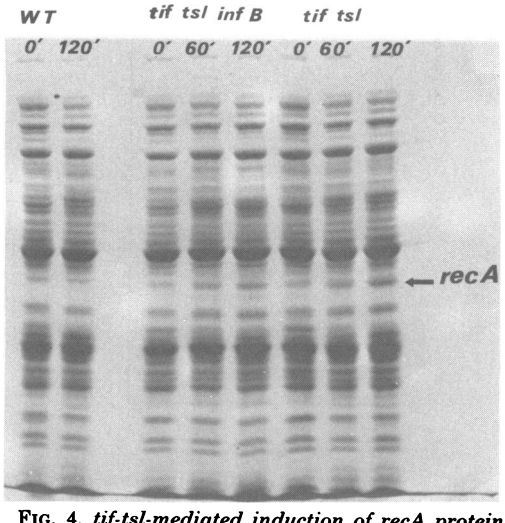

FIG. 4. *tif-tsl-mediated induction of recA protein synthesis. Exponential-phase cultures growing in N medium at 30°C were diluted into NA medium and shifted to 42°C at zero time. Samples were withdrawn at 0, 60, and 120 min. Protein was extracted and analyzed on a sodium dodecyl sulfate-polyacrylamide gel as described in the text. The strains used were GC4148 (tif-1 tsl-1), GC4191 (tif-1 tsl-1 infB1), and JM1 (WT).*

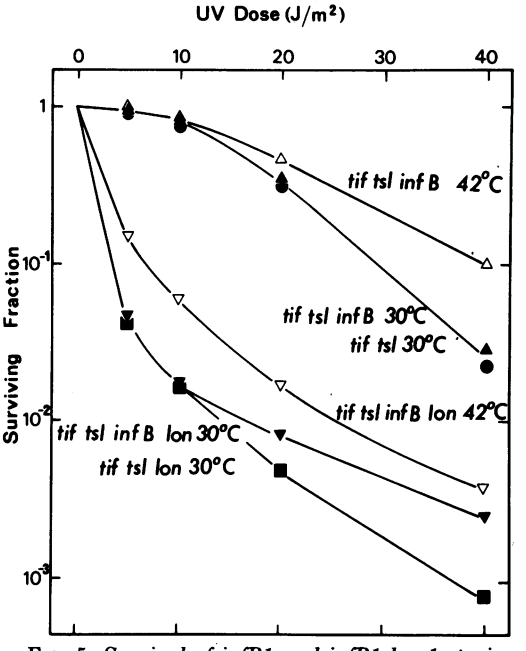

FIG. 5. *Survival of infB1 and infB1 lon-1 strains after UV irradiation. Exponential-phase cultures growing in LB broth at 30°C were centrifuged, suspended in 10^{-2} M $MgSO_4$, irradiated or not treated, and plated onto LB medium plates which were incubated at 30°C (closed symbols) or 42°C (open symbols). The strains used were GC4148 (tif tsl) (●), GC4191 (tif tsl infB) (▲ and △), GC4336 (tif tsl lon) (■), and GC4325 (tif tsl infB lon) (▼ and ▽).*

(iii) Inhibitory effect of the *infB1* mutation on *tif*-mediated λ induction and bacterial mutagenesis. In an attempt to define more precisely the degree of pleiotropy conferred by the *infB1* mutation, we examined its effect on λ prophage induction and bacterial mutagenesis, either by *tif* expression or after UV irradiation.

The induction of a λ prophage by expression of the *tif-1* mutation can be observed conveniently by monitoring the optical density of a culture in EMMA at 40°C (8). A comparison of cultures of *tif-1 tsl-1*(λ) and *tif-1 tsl-1 infB1*(λ) strains under these conditions showed (Fig. 6) that both cultures lysed but that lysis was retarded and less complete in the *infB1*(λ) culture. The sluggish character of this induction was further illustrated by the fact that the efficiency of colony formation on EMMA plates at 40°C was 1 for the *tif-1 tsl-1 infB1*(λ) strain, compared with 8×10^{-5} for the *tif-1 tsl-1*(λ) strain.

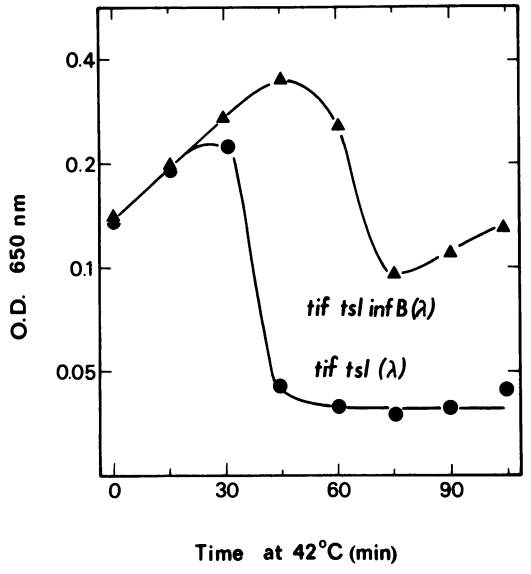

FIG. 6. *Interference with tif-mediated λ induction by the infB1 mutation. Exponential-phase cultures growing in EMM at 30°C were diluted into prewarmed EMMA and shifted to 42°C at zero time. Samples were withdrawn periodically for measurements of optical density at 650 nm (O.D. 650 nm). The strains used were GC4148(λ) [tif tsl(λ)]* (●) *and GC4191(λ) [tif tsl infB(λ)]* (▲).

UV induction of λ was studied in the same lysogens, *tif-1 tsl-1*(λ) and *tif-1 tsl-1 infB1*(λ). The percentage of cells forming infective centers (measured at 30°C as a function of UV dose) was indistinguishable for the two strains, reaching 50% at a dose of 35 J/m^2 (data not shown).

The mutator effect of the *tif-1* mutation can be evaluated quantitatively in *tif sfi* strains by comparing the reversion frequency of the *his-4* ochre mutation at 30°C to the reversion frequency at 40°C on histidine-limiting plates containing adenine (15). We carried out such a comparison in nearly isogenic *his-4*, *tif-1 tsl-1 sfiA11 his-4*, and *tif-1 tsl-1 infB1 his-4* strains. Table 4 shows that the mutator effect of the *tif-1* mutation was clearly reduced by the presence of the *infB1* mutation but was not totally suppressed.

UV-induced reversion of the *his-4* ochre mutation in the same strains was measured at 30 and 42°C on semienriched minimal medium plates supplemented with guanosine and cytidine, conditions under which *tif*-induced mutagenesis at 42°C was completely inhibited. The *infB1* strain was clearly UV mutable at doses of 20 and 40 J/m^2. No significant difference was observed between 30 and 40°C or between the *infB1* and *sfiA11* strains at either temperature (data not shown).

DISCUSSION

The filamentation induced by the expression of the *tsl* and *tif* mutations requires a protein whose synthesis is induced under these conditions; in *tsl* strains, a low concentration of rifampin restores normal cell division at 42°C (34), and in *tif* strains the presence of chloramphenicol during the 40°C induction period prevents subsequent filamentation at 30°C (21a). We have tentatively assumed that this inducible protein is the hypothetical repair-associated division inhibitor proposed by George et al. (15).

To account for the similar induction patterns of the filamentation response and *recA* protein synthesis, Satta and Pardee (34) suggested that the SOS-associated division inhibitor might be the *recA* protein itself. The present work indicates that this is not the case. In a direct selection for suppression of *tsl-tif*-induced filamentation, no *recA* mutants were found ($\leq 10^{-8}$ for UV-sensitive revertants; $\leq 10^{-7}$ for UV-resistant revertants). Furthermore, in a *tsl-1 zab-53* strain the amount of *recA* protein synthesized at 42°C remains at the low basal level visible at 30°C (24; unpublished data), yet cell division is completely arrested (Fig. 1). Indeed, the *recA* protein does not seem to have any direct role in the septation block, for not even the basal level of *recA* protein can be detected in a *tsl-1 recA99 sup*$^+$ strain at 42°C (23; unpublished data), whereas cell division is completely arrested (Fig. 1).

Massive synthesis of the *recA* protein and cell division arrest (filamentation) are dissociated in the *tsl-1 zab-53* strain, although the *recA* product is not eliminated as in *recA99* strains. Since the *zab-53* mutation is at the *recA* locus (10), this dissociation has been interpreted as evidence that *zab-53* affects the *recA* promoter (24). This hypothesis implies that the division inhibitor gene is not located in the *recA* operon.

The opposite type of dissociation is observed

TABLE 4. *Effect of the infB1 mutation on tif-mediated mutagenesis*[a]

Strain	Relevant genotype	No. of His$^+$ colonies per plate at:[b]	
		30°C	40°C
AB1157	*his-4*	6	15
GC4259	*tif-1 tsl-1 sfiA11 his-4*	6	215
GC4191	*tif-1 tsl-1 infB1 his-4*	11	23

[a] Exponential-phase cultures growing in EMM at 30°C were centrifuged and suspended in M63 medium. Approximately 10^7 cells were plated onto histidine-limiting (0.8 μg/ml) plates and incubated at 30 or 40°C.

[b] Mean of duplicate platings.

in the *tsl-1 sfiA11* strain, which induces massive synthesis of *recA* protein at 42°C but does not filament. The *sfi* mutations are specific suppressors of SOS filamentation, whether they are induced by *tsl* or *tif* expression or by UV irradiation, and they do not affect the induction of other aspects of the SOS response. The dissociation observed is thus best explained by the hypothesis that the *sfi* mutations inactivate the division inhibitor or its site of action.

The *infB1* mutation, like *sfiA11* (and *sfiB114*), suppresses *tsl-tif*-induced filamentation but not *recA* protein synthesis. The *infB1* mutation, however, differs in several important respects from *sfiA11* (and *sfiB114*). (i) It does not suppress UV-induced filamentation in a *lon-1* derivative, and (ii) it retards or reduces *tif*-mediated induction of λ prophage, of bacterial mutagenesis, and (probably) of the *recA* protein. This pleiotropic phenotype strongly suggests that the *infB1* mutation defines a regulatory gene involved in *tif* and *tsl* expression. If this is the case, then it follows that the regulation of the *recA* gene is not strictly identical to the regulation of the division inhibitor gene.

During the preparation of this paper, Darby and Holland (11) reported experiments indicating that UV-induced division inhibition in *lon* and *lon*$^+$ strains exhibits kinetics and rifampin sensitivity which are different from the kinetics and rifampin sensitivity observed for *recA* protein synthesis. They also conclude that de novo synthesis of *recA* protein is not necessary for cell division arrest.

Several other mutations in the 66- to 83-min region of the *E. coli* genetic map have been reported to affect the SOS response. The *recF143* mutation has been shown to retard the onset of derepression of λ prophage after UV irradiation or mitomycin C treatment (2). The *mul* mutation in *recA*$^+$ strains increases the frequency of clear mutants among survivors of UV-irradiated λ phage (36). The *dnaA46* mutation interferes with *tif*-mediated induction of λ prophage (12). All of these mutations, however, are cotransducible with *ilv* (*recF143* at 2.5% [20], *mul* at 3.5% [36], and *dnaA46* at 7% [38]); the absence of cotransduction between *infB1* and *ilvA* (0 of 240) may indicate that the *infB* locus is different from *recF*, *mul*, and *dnaA*.

The *inf-3* mutation, which was selected as a suppressor of λ prophage induction by thymine starvation (13), has properties similar to those of *infB1* (4); it is located in the 66- to 83-min region, it suppresses *tif*-mediated filamentation, and it does not affect DNA repair capacities (UV-resistant phenotype). Unlike *infB1*, however, the *inf-3* mutation also suppresses induction of λ by *tif* expression or after UV irradiation. It is possible that the *inf-3* and *infB* mutations affect the same locus, the phenotypic differences between the two mutants being strain or allele specific. More precise mapping of the two mutations should clarify their relationship and permit complementation tests between them. Meanwhile, we have chosen to use the name *infB* here, and we suggest that the locus analyzed by Bailone et al. (4) be called *infA*.

Current models to account for the regulation of the SOS response are based on observations showing that the induction of λ prophage is accompanied by proteolytic cleavage of the λ repressor (32) and that this cleavage can be produced in vitro by the purified *recA* product (33). Several authors have proposed (14, 18, 23) that the various SOS functions are negatively controlled by repressors resembling the λ repressor; these repressors are cleaved by an activated (protease) conformation of the *recA* protein, *recA'*, which in turn is formed by an interaction of "normal" *recA* molecules with an effector molecule generated when DNA replication is perturbed. To explain the LexA$^-$ and Tsl$^-$ phenotypes, these same authors propose that the *lexA*$^+$ gene product is the repressor of the *recA* gene (and implicitly of the division inhibitor gene), cleaved by the protease *recA'*. A recent report (35) suggested that the induction of massive synthesis of the *recA* product may not be due to the same type of proteolytic cleavage as that resulting in λ induction. To account for this observation, modifications of the above model have been proposed; according to these modifications the molecular mechanism of *recA* induction is different from the mechanism of λ induction (5, 35). Our results with the *infB1* strains suggest that the induction of division inhibitor synthesis may be different from both *recA* induction and λ induction.

The regulation of the SOS filamentation response (synthesis of the division inhibitor) in *lon* strains has previously been shown to differ from the regulation of other aspects of the SOS response. Irreversible filamentation is induced in these strains at UV doses too low to induce λ prophage (7, 22). Filamentation is also induced after a nutritional shift-up (37, 21a), whereas λ prophage is not.

A function as important as a potentially lethal division inhibitor is almost certain to be tightly controlled. It is not unreasonable to speculate that several different regulatory mechanisms may govern its synthesis. Work is in progress to elucidate the roles of the various functions already known to affect the filamentation response (*recA*, *lexA*, *sfiA*, *sfiB*, *infA*, *infB*, *lon*) and to

identify other loci involved in induction or expression of SOS filamentation.

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