JOURNAL OF BACTERIOLOGY, Oct. 1981, p. 265-273
0021-9193/81/100265-09$02.00/0

Vol. 148, No. 1

Role of *sulA* and *sulB* in Filamentation by Lon Mutants of *Escherichia coli* K-12

SUSAN GOTTESMAN,* ETHAN HALPERN, AND PATSY TRISLER

Laboratory of Molecular Biology, National Cancer Institute, Bethesda, Maryland 20205

Received 15 January 1981/Accepted 29 June 1981

Cells containing the pleiotropic *Escherichia coli* mutation *lon* filament extensively and die after exposure to ultraviolet light. Outside suppressors of the ultraviolet sensitivity, called *sul*, have previously been described at two loci; these mutations reverse the ultraviolet sensitivity of *lon* strains but do not affect the mucoidal or degradation defect of these strains. An isogenic set of strains carrying combinations of *lon*, *sulA*, and *sulB* was constructed, and their behavior during normal growth and after ultraviolet treatment was studied. *sulA* mutations had no detectable phenotype in lon^+ cells; the *lon sulA* strains filamented transiently after ultraviolet irradiation, as did lon^+ sul^+ cells. We found that the *sulB* mutation, which alters cell morphology and slows recovery from transient filamentation after ultraviolet treatment, was epistatic to both *lon* and *sulA*. Whereas *sulA* mutations were recessive to the wild-type allele, *sulB* was partially dominant. The simplest model to account for our observations is that *sulA* and *lon* participate in a pathway of filamentation independent of that which produces transient filamentation in wild-type strains; *sulB* product may be the target of *sulA* action and may play a role in normal cell division.

Escherichia coli lon mutants are defective in a number of cellular processes. The mutants show decreased degradation of missense proteins and nonsense proteins (8, 17) as well as certain wild-type proteins (7). They filament extensively after treatment with UV light (9) and overproduce capsular polysaccharide (15). In addition, *lon* cells are defective in plasmid maintenance and establishment (3, 18) and λ lysogeny (19).

We have been examining mutations, called *sul* (14), which can be isolated as UV-resistant or methyl methane sulfonate (MMS)-resistant derivatives of *lon* (5, 13, 14). *sul* mutations are located at two loci on the *E. coli* chromosome: *sulA*, near *pyrD*, and *sulB*, near *leu* (5, 13). Mutations with similar phenotypes, *sfiA* and *sfiB*, have been isolated in the same regions as temperature-resistant revertants of a *tif lon* double mutation (6). These *tif lon* strains filament extensively at a high temperature; the *sfi* survivors show less filamentation. We will assume in our discussion that the *sulA* and *sfiA* mutations are allelic and that *sulB* and *sfiB* are allelic, since their phenotypes are indistinguishable and they map in the same regions of the chromosome (6, 12, 13, 14).

In this work, we have examined the question of whether the *sulA* and *sulB* genes have any role in the normal process of cell division or act only after treatment with UV light or MMS as part of the induced SOS system (16, 20). We have genetically analyzed the interaction of *lon*, *sulA*, and *sulB*. We find that *sulA* affects only the abnormal filamentation shown by *lon* strains after UV treatment and that all *lon* effects on filamentation are mediated through *sulA*. *sulB* mutations are epistatic to both *lon* and *sulA* and may be involved directly in cell division. Possible models for the interaction of these genes are considered.

MATERIALS AND METHODS

Bacterial and phage strains. Bacterial strains are described in Table 1. λ *c*I857 was obtained from the National Institutes of Health phage collection, and λ *c*I8570ts was obtained from N. Kleckner. λ *c*I857 *N*ts *O*ts was obtained from a cross between *imm*434 *N*ts and λ *c*I857 *N*am7 *N*am53 *O*ts21 *gal*8 (both from N. Kleckner).

Media and buffers. Broth for growing cells in liquid and on plates has been described previously (8). For growth of bacteria in low salt, luria broth was made by using 1/10th the normal amount of NaCl, 0.5 g/liter of water.

Strain construction. Procedures for P1 transductions and lysates have been described previously (8). The particulars of strain construction are summarized in Table 1.

lon is usually introduced into strains by cotransduction with *proC*. Use of the F *gal*(Ts) in constructing nonmucoid *lon* strains was described by Gottesman

TABLE 1. *Bacterial strains*

Strain name	Genotype or allele	Reference (source) or construction steps
A. Isogenic *lon* non-mucoid[a]		
Parental		
SG4030	F⁻ *proC* Δ(*gal-att-bio*) *thi rpsL*	8
SG4038	F⁻ *proCYA221* Δ(*gal-pgl*)*324 thi rpsL*	P1(SA494; Δ[*gal-pgl*]) to SG4030; select *bio*⁺ (4)
SG4424	F′ *gal*/*proC* Δ(*gal-pgl*)*324 thi rpsL*	F′*gal* in SG4038
lon		Source for P1 lysate
SG4044	Δ*lon-100*	SA1500 (8)
SG4045	*capR6*	SA1384
SG4046	*lonT2*	DT521 (2)
SG4047	*lonR1*	HR521 (1)
SG4058	*lon-300*	JF66 from J. Falkinham
SG4059	*lon-21*	PAM153 from J. Falkinham
SG4060	*lon-6*	AB1896 from J. Falkinham
SG4061	*lon-1*	JF74 from J. Falkinham
SG4063	*lon-9*	MC102 from J. Falkinham
SG4064	*lon-7*	AB1897 from J. Falkinham
SG4065	*lon-10*	M6 from J. Falkinham
B. Isogenic *lon sul*[b]		
SG13008	F⁻ *his pyrD rpsL*	SA734 from S. Adhya
SG13009	F⁻ *his pyrD* Δ*lon-100 rpsL*	P1(SA1500) to SG13008; screen for mucoid
SG13022	F⁻ *his pyrD* Δ*lon-100 leu*::Tn*10 rpsL*	P1(*leu*::Tn10) (from H. Miller) to SG13009
SG13082	F⁻ *his leu*::Tn*10* Δ*lon-100 pyrD*⁺ *sulA366 rpsL*	P1(EJ366) to SG13022; select *pyr*⁺, screen for MMS^r
SG13083	F⁻ *his pyrD leu*⁺ *sulB367* Δ*lon-100 rpsL*	P1(EJ367) to SG13022; select *leu*⁺, screen for MMS^r
SG13091	F⁻ *his sulA366 sulB367* Δ*lon-100 rpsL*	P1(13083) to SG13082, select *leu*⁺, screen by back-transduction to SG13022
SG13092	F⁻ *his sulA366 sulB367* Δ*lon-100 rpsL*	P1(SG13082) to SG13083; select *pyr*⁺; screen by back-transduction to SG13022
SG13107	F⁻ *his pyrD leu*::Tn*10 rpsL*	P1(SG13082) to SG13008; select tetracycline resistance
SG13109	F⁻ *his sulA366 leu*::Tn*10 rpsL*	P1(SG13082) to SG13107; select *pyr*⁺ and screen by back-transduction to SG13022
SG13110	F⁻ *his sulB367 pyrD rpsL*	P1(SG13083) to SG13107; select *leu*⁺ and screen by back-transduction to SG13022
SG13400	F′104/*his leu*::Tn*10 pyrD* Δ*lon-100 rpsL*	KLF4/AB2463[c] × SG13022
SG13401	F′126/*his leu*::Tn*10 pyrD* Δ*lon-100 rpsL*	KLF26/KL181[c] × SG13022
SG13085	F⁻ *his leu*::Tn*10 pyrD* Δ*lon-100 sulA rpsL*	Spontaneous MMS^r derivative of SG13022; *sulA* confirmed by P1 transduction of this strain to *pyrD*⁺, loss of MMS^r
SG13095	F⁻ *his pyrD sulB367 thr*::Tn*10 rpsL* Δ*lon-100*	P1(SG2205) to SG13083; select tetracycline resistance (SG2205 contains *thr*::Tn*10*)
SG13402	F′104/*his leu*::Tn*10 pyrD sulA rpsL* Δ*lon-100*	KLF4/AB2463 × SG13085
SG13403	F′126/*his pyrD sulA leu*::Tn*10 rpsL* Δ*lon-100*	KLF26/KL181 × SG13085
SG13404	F′104/*his pyrD sulB367 thr*::Tn*10 rpsL* Δ*lon-100*	KLF4/AB2463 × SG13095
SG13405	F′126/*his pyrD sulB367 thr*::Tn*10 rpsL* Δ*lon-100*	KLF26/KL181 × SG13095

[a] All strains in this group were constructed by P1CMclr100 transduction of SG4424 to *pro*⁺. Mucoid, MMS-sensitive transductants were purified and a *gal* segregant was obtained by streaking the strain at 39°C on a galactose MacConkey agar plate.

[b] All strains described here were made for this work; they all derive from SG13008 (SA734):F⁻ *his pyrD rpsL*.

[c] KLF4/AB2463 and KLF26/KL181 were obtained from B. Bachmann; they are CGSC numbers 4251 and 4253, respectively. F′104 covers the *thr-argF* region; F′126 covers *nadA-rac*, including *trp* and *pyrD*.

and Zipser (8). *sulA* was introduced by cotransduction with *pyrD*, and *sulB* was introduced by cotransduction with *leu*. In cases where the phenotype of the *sulA* or *sulB* marker is not known or might not be easily detectable (i.e., construction of *sulA sulB* double mutants; *sulA* and *sulB* in *lon*⁺ strains), a number of independent transductants were purified. P1 grown on these transductants was used to transduce SG13022 (Δ*lon-100 leu pyrD*; MMS sensitive) to either *leu*⁺ or *pyrD*⁺. In this strain, transductants were screened for MMS resistance. Thus, if the original strain carried a *sul* marker linked to *leu*, we would expect P1 grown on that strain to yield Leu⁺ MMS-resistant transductants of SG13022.

Two independent *lon sulA sulB* strains were constructed (SG13091 and SG13092). Both strains acted identically in all tests.

UV light treatment and dark-phase microscopy. Cells were grown in LB to 2×10^8 bacteria per ml at 37°C, centrifuged in a Beckman model J-21B centrifuge at 5,000 rpm for 10 min, and suspended in 0.1 volume of 0.01 M $MgSO_4$. A large drop of the suspended culture was placed on a plastic petri dish and irradiated at a rate of 28 ergs/mm^2 per s for 20 s. (An inducing dose for λ with this UV lamp is 13 s.) In dim light, the cells were diluted 50-fold into LB broth and incubated further at 37°C. Control cultures were centrifuged, resuspended in $MgSO_4$, and diluted back into LB without UV irradiation.

To prepare the cells for microscopy, they were collected by centrifugation at 5,000 rpm for 10 min, suspended in 0.1 volume of 0.01 M $MgSO_4$, and smeared on a slide. After allowing the slide to dry for 1 to 2 min, the slide was submerged in ethanol. The cells were viewed by using dark-field microscopy at ×400 enlargement with a Zeiss phase-contrast microscope and photographed.

For measurement from photographs of the length of filaments, all bacteria with two clearly defined ends were measured with an electronic graphic calculator (Numonics Corp.). We have observed clumps of filaments with the *lon* strains; if filaments preferentially clump, our estimate of the proportion of filaments in a given culture may be low.

RESULTS

Isolation of *sulA* and *sulB* mutations. Although the *lon* mutation exerts broadly pleiotropic effects on the cell, one can separate these effects genetically by isolation and characterization of specific mutations outside the *lon* locus which affect some part of the *lon* pathway. It has previously been reported that MMS-resistant suppressors of *lon* (*sul*) can be isolated at two sites on the *E. coli* chromosome, near *pyrD* and *leu* (5, 13). We found that MMS-resistant derivatives of a presumed *lon* point mutation fell into two classes: (i) mucoid and defective for degradation (these strains could be demonstrated to still carry the original *lon* mutation as well as a suppressing mutation at another location) or (ii) nonmucoid, with normal protein degradation (these strains had lost the original *lon* allele and presumably represented reversion at the *lon* locus [8]).

We extended this analysis by isolation of more than 100 MMS-resistant derivatives of a *lon* deletion strain from 10 independent cultures. All revertants to MMS resistance remained mucoid and defective for protein degradation; 10 revertants tested for the presence of *sul* mutations (by P1 transduction to a *lon pyrD leu* recipient) all contained *sulA* mutations. These results support the assumption that SG4044, the strain used for these reversion experiments, carries a deletion of the *lon* gene and suggest that the pathways for *lon* effects on filamentation do not include effects on mucoidy and protein degradation.

To isolate a large number of *sulA* and *sulB* mutations for further study, 10 isogenic strains carrying different *lon* alleles (originally isolated for a variety of phenotypes in different strain backgrounds) were plated on MMS plates. MMS-resistant derivatives were pooled, and the pooled cells were infected with P1. The resulting P1 lysates were used to transduce a recipient, SG13022 (*Δlon-100 pyrD leu*), selecting either Leu$^+$ or Ura$^+$ colonies. Leu$^+$ and Ura$^+$ transductants were screened for MMS resistance (Table 2). Previous work in this laboratory and others has indicated that *sulA* cotransduction with *pyrD* is 55%, whereas *sulB* and *leu* are 47% linked by P1 transduction. From the observed contransduction of MMS resistance and the selected markers, we estimated that approximately 6 of 10 of our MMS-resistant bacteria were *sulA* mutations, whereas approximately 1 of 10 carried a *sulB* mutation. Because we were able to detect *sul* alleles for approximately 7 of 10 of the MMS-resistant donor strains, most *sul* mutations, selected in strains carrying a variety of *lon* alleles, were able to suppress the *lon* deletion mutation in our transduction recipient. Some of the remaining MMS-resistant bacteria may represent revertants at the *lon* site; if other *sul* loci exist, we would not have detected them in these experiments.

Properties of *sul* mutations: UV sensitivity and filamentation. Lon$^+$ strains filament transiently after treatment with UV or radiomimetic agents, such as MMS. In contrast, Lon$^-$ strains continued to filament indefinitely after UV treatment and were sensitive to UV light. UV-resistant derivatives of *lon* strains may carry either the *sulA* or the *sulB* mutation; these *lon sul* double mutations do not form extended filaments after induction of the SOS system (5, 6, 11). Similarly, *sfiA* and *sfiB* mutations block the formation of filaments after UV treatment of a *tif lon* strain. Neither *sfiA* nor *sfiB* affects other characteristics of UV repair or induction of SOS functions (6, 12). Thus, one can conclude that filamentation is in fact the lethal event in *lon* mutant cells treated with UV light. The model proposed by George et al. (6) suggests that a septation inhibitor, coded for by *sulA* or *sulB* or both, is responsible for the transient filamentation seen in *lon*$^+$ cells. In *lon* cells, the filamentation persists, possibly because proteolysis of the septation inhibitor is abnormally slow. If this is so, mutations in *sulA* and *sulB* would be expected to abolish transient filamentation in wild-type cells and to prevent the more dramatic

TABLE 2. *Isolation of sulA and sulB mutations*[a]

Transductants	Total no.	No. of MMS-resistant transductants	Apparent cotransduction (%)	Actual cotransduction (%)	Frequency in pool (%)
Ura$^+$	69	24	35	55	64
Leu$^+$	69	3	4.3	47	8.5

[a] P1 lysates grown on pooled MMS-resistant transductants from each of nine *lon* mutants were used to transduce SG13022 (Δ*lon leu pyrD*) to either Leu$^+$ or Ura$^+$. Transductants were screened for MMS resistance. The actual cotransduction values were obtained by transducing from a purified MMS-resistant Leu$^+$ or Ura$^+$ transductant back into SG13022, selecting Leu$^+$ or Ura$^+$ transductants, and screening once again for MMS resistance. All nine *lon* alleles gave MMS-resistant colonies at a frequency of 10^{-5} to 10^{-6} on LB plates containing MMS (0.05%). Seven *lon* alleles gave rise to *sulA* mutations; three gave rise to *sulB* mutations.

filamentation seen in *lon* cells.

To assess the effects of *sul* mutations on filamentation, a set of isogenic *lon*$^+$, *lon*, *lon sulA*, *lon sulB*, *lon*$^+$ *sulA*, *lon*$^+$ *sulB*, and *lon sulA sulB* strains was constructed. The construction of these strains is described in Materials and Methods and in Table 1. The presence of the appropriate *sul* locus was confirmed by P1 transduction of the *sul* region to SG13022 (F$^-$ *pyrD leu lon*; see Materials and Methods).

Wild-type and mutant strains were grown in LB broth, treated with UV light, allowed to continue growth in LB broth, and examined by dark-field microscopy. Since we were interested in determining the effects of *sul* mutations on transient filamentation, we examined the cells at relatively early times after UV treatment. Figure 1 shows the time courses of filamentation in *lon*$^+$ and *lon* cells. At 1 to 2 h after treatment with UV light, cells in both the *lon*$^+$ and the *lon* strains were somewhat filamented. By 4 h, however, the *lon*$^+$ cells had recovered from this transient filamentation and resumed normal growth. In contrast, the *lon* cells had not regained their normal morphology. Longer filaments could be observed as growth continues, but septation did not resume. We compared cells containing *sulA* and *sulB* mutations to their parents 2 h after UV treatment, when transient filamentation was most obvious (Fig. 2 and Table 2). We observed that neither *sulA* nor *sulB* mutations abolished transient filamentation. Therefore, we concluded that the *sulA*$^+$ and *sulB*$^+$ products are not necessary for transient filamentation.

The other aspect of the working model, that *lon* cells are UV and MMS sensitive because of their exaggerated filamentation after UV treatment, can be tested by examining the behavior of UV-resistant *sul lon* strains at times after UV treatment when wild-type (UV-resistant) cells have recovered from their transient filamentation. At 4 h after UV treatment, *sulA lon* strains had in fact returned to normal growth and were indistinguishable from *lon*$^+$ strains (Table 3). Therefore, for the *sulA* mutation, the prediction that filamentation and lethality are linked is supported. For *sulB* mutations, the situation is more complex; 4 h after UV treatment, *lon sulB* cells still showed significant numbers of filaments. However, at later times after UV treatment, *sulB lon* strains were not so filamentous and were easily distinguishable from *lon sul*$^+$ strains under the microscope. The *sulB* strains did not form the very long filaments characteristic of the *lon* strain at any time after UV treatment. Thus, both *sul* loci that confer UV resistance on *lon* strains also blocked the extended filamentation without blocking transient filamentation.

***sulB* mutations are epistatic to *lon* and *sulA*.** Our studies of *lon sul* and *lon*$^+$ *sul* strains suggested that strains carrying *sulB* mutations were abnormal in a number of respects. *lon* cells carrying the *sulB* allele were abnormally long even before treatment with UV light (Table 3) and seemed to take longer to recover from transient filamentation after UV treatment (Table 3 and unpublished data). These unique characteristics of *sulB* strains allowed us to look at the interactions of *sulB* with *lon* and *sulA* by examining the behavior of the appropriate double and triple mutations. We found in all cases that the *sulB* mutation was epistatic to *lon* and *sulA*. Thus, the abnormally long cells characteristic of the *sulB* mutant strain were seen in *sulB lon* and *sulB lon sulA* cells (Table 3). Similarly, the delayed recovery from filamentation, measured as the presence of filaments 4 h after UV treatment and which disappeared with continued growth of the cells, was found in strains carrying the *sulB* allele (Table 3). Therefore, these peculiarities of *sulB* behavior were not affected by the state of the *lon* or *sulA* genes.

Johnson (13) found that a *sulB lon* strain grew abnormally in rich broth; his *sulB* allele was temperature and salt sensitive for growth and filamented extensively in low-salt medium. Johnson did not present data for the *sulB* locus in the absence of a *lon* mutation. We have

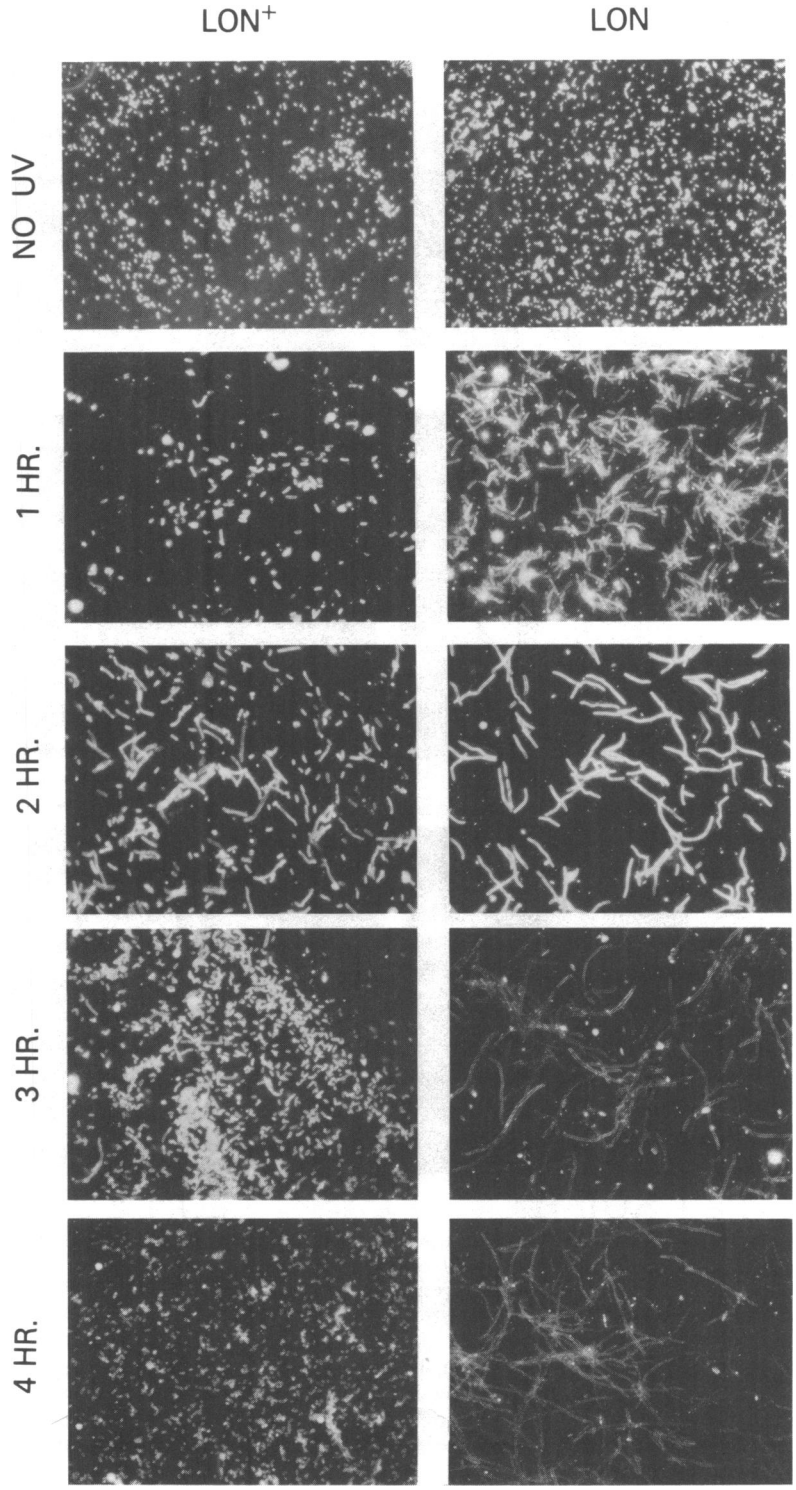

FIG. 1. *Time course of filamentation of lon+ and lon cells after UV treatment. Strains SG13008 and SG13022 were grown in LB broth, concentrated in $MgSO_4$ buffer, and irradiated as described in the text. Cells were resuspended at their original density in LB broth and grown in the dark at 37°C. Samples were removed at 1, 2, 3, and 4 h and examined by dark-phase microscopy as described in the text. Control cultures were concentrated in buffer and resuspended without UV irradiation.*

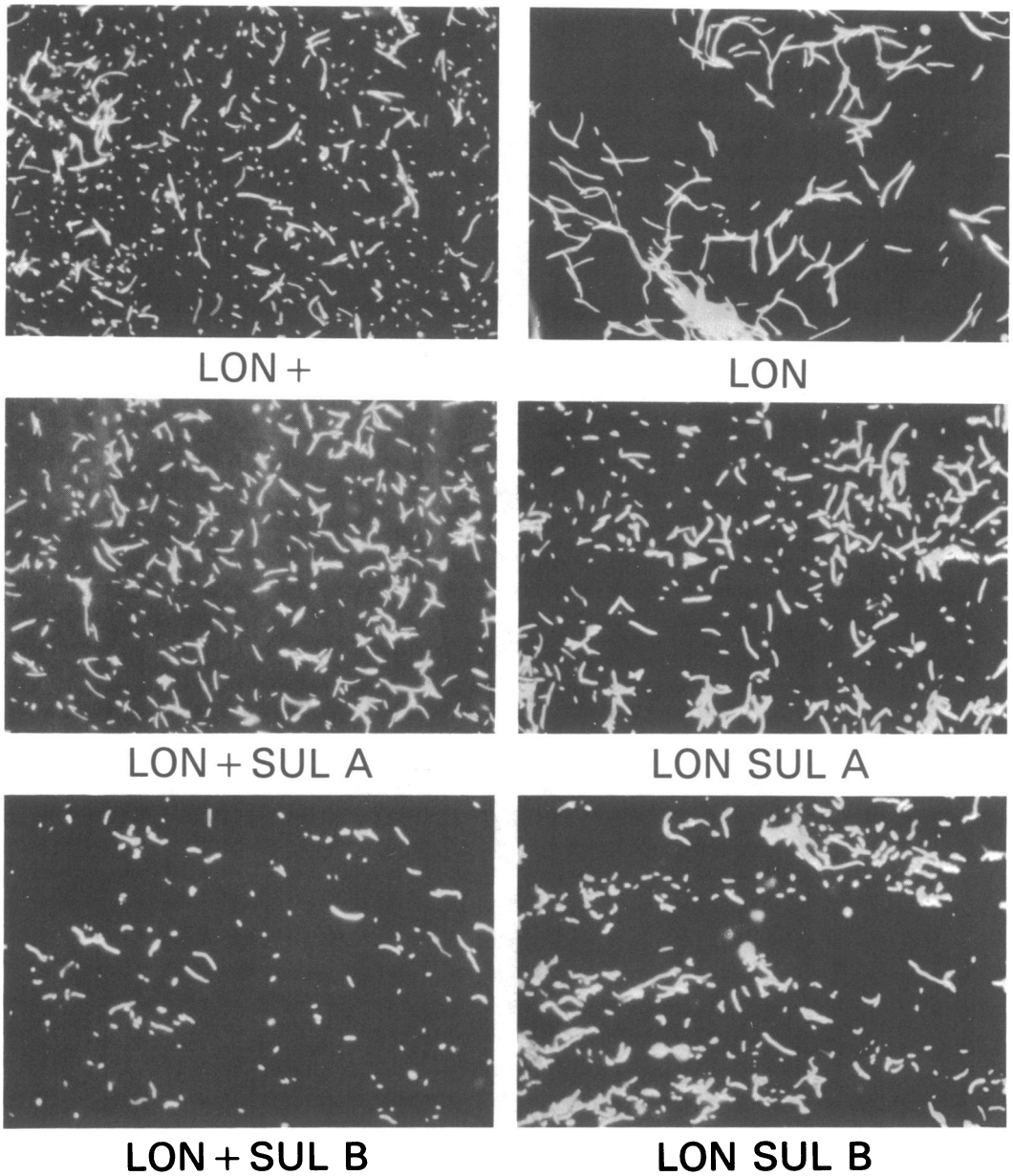

FIG. 2. *Transient filamentation in Lon Sul strains 2 h after UV treatment. Strains SG13107 (lon⁺), SG13022 (Δlon), SG13082 (Δlon sulA), SG13083 (Δlon sulB), SG13109 (lon⁺ sulA), and 13110 (lon⁺ sulB) were grown in LB, UV irradiated, and allowed to continue growth as described in the text. Cells were removed at 2 h, collected, and examined by dark-phase microscopy.*

confirmed this result in a *sulB lon*⁺ strain; in rich broth containing 1/10th the normal concentration of NaCl, at both low (30°C) and high (40°C) temperatures (Table 4), the strain filamented extensively. Our strain showed only a slight temperature dependence in this response.

***sulB* mutations are dominant.** Diploid derivatives of our *lon*, *lon sulA*, and *lon sulB* strains, carrying either F'104 (*thr*⁺ *leu*⁺) or F'126 (*pyr*D⁺), were constructed as described in Materials and Methods (Table 1). The diploid strains and their haploid parents were examined for MMS resistance and filamentation after UV treatment. The results indicate that, as previously reported (5, 12), *sulA* mutations are generally recessive to the wild type (Table 5). The

sulB mutation, however, is dominant to wild type.

To confirm that the F'104 episome does indeed carry a *sulB*$^+$ allele, we grew P1 on the diploid *sulB*$^+$/*sulB*367 strain and transduced a recipient *leu sulB lon* strain, selecting Leu$^+$ transductants and scoring for MMS resistance (Table 6). Whereas P1 grown on a haploid *leu*$^+$ *sulB* strain gave no MMS-sensitive transductants, *sulB*$^+$ strains gave 45 to 50% MMS-sensitive recombinants in control transductions. P1 grown on the diploid gave transductants, 19% of which were MMS sensitive (Table 5). Since there are two *leu*$^+$ genes in this strain, one next to *sulB367* and the other next to *sulB*$^+$, this frequency is almost precisely what one might expect of a diploid of the proper constitution. The F'104 episome was introduced into strains carrying five independent *sulB* alleles. In all cases, the resulting diploid was still MMS resistant and formed the medium-length filaments after UV treatment characteristic of *sulB* mutations.

TABLE 3. *Cell lengths after UV treatment*[a]

Genotype	Measured cell length (no UV treatment)	Relative cell length after UV at:	
		2 h	4 h
lon$^+$	0.1	1.9	1.2
lon	0.09	5.9	5.9
lon$^+$ *sulA*	0.10	2.6	1.0
lon$^+$ *sulB*	0.10	3.4	1.0
lon sulA	0.08	2.5	1.1
lon sulB	0.16	2.1	1.7
lon sulA sulB	0.19		1.3

[a] Cell lengths are normalized to lengths of cells from the same culture, not exposed to UV light but otherwise treated identically to the UV-treated culture. At least 20 cells for each time point were measured from photographs, as described in the text.

TABLE 4. *Filamentation of sulB strains in low-salt medium*[a]

Strain	Temp of broth (°C)	Cell length in:	
		Normal	Low-salt
lon$^+$ *sulB*$^+$	30	0.11	0.15
	40	0.12	0.14
lon$^+$ *sulB*	30	0.17	0.37
	40	0.14	0.43

[a] Strains SG13107 and SG13110 were grown in LB (5 g/liter of NaCl) and LB with ½0th the normal NaCl concentration (0.5 g/liter). Cell lengths are arbitrary units derived from measuring cells on photographs; wild-type cells grown in LB average about 0.1 units.

DISCUSSION

We have found that both *sulA* and *sulB* mutations block the formation of abnormally long filaments in *lon* strains but do not block the transient filamentation exhibited by strains within a few hours after UV treatment. This observation suggests the existence of two filamentation systems, both activated after UV treatment. The first, which produces transient filamentation (observable 1 to 3 h after UV treatment), is independent of the state of the *sulA, sulB*, and *lon* loci. The second, which we will call *sul*-dependent filamentation (present in *lon sul*$^+$ cells), leads to irreversible filamentation after UV treatment and is responsible for the UV-sensitive phenotype of *lon* strains. Huisman et al. (11, 12), as a result of their observation of thymine starvation-induced filamentation in *sfi* strains, have also suggested the possibility of two filamentation systems. They found that *sfi* mutations abolish rapid filamentation after thy-

TABLE 5. *Properties of F'/sul*$^-$ *diploid strains*[a]

Genotype	Surviving fraction on MMS plates[b]	Filamentation	
		Without UV	With UV
Δ*lon sul*$^+$ *leu pyrD*	$<10^{-4}$	None	Long
F'104/Δ*lon sul*$^+$	$<10^{-4}$	Few	Many, long
F'126/Δ*lon sul*$^+$	$<10^{-5}$	None	Medium to long
Δ*lon sulA leu pyrD*	1.0	None	Few, small
F'104/Δ*lon sulA*	1.0	None	Few, small
F'126/Δ*lon sulA*	2×10^{-5}	None	Many, long
Δ*lon sulB thr pyrD*	1.0	Few, small	Some, medium
F'104/Δ*lon sulB*	0.38	Few, small	Many, medium
F'126/Δ*lon sulB*	0.13	Few, small	Many, medium

[a] The isogenic set of strains SG13022, SG13085, and SG13095 was used, as well as the F' derivatives of these strains described in Table 1. Cells were tested for the presence of the episome from the cultures used for the UV experiments by assaying for *leu*$^+$, *thr*$^+$, or *trp*$^+$ cells. In all experiments, greater than 90% of the cells retained the episome at the time of UV treatment.

TABLE 6. *Rescue of sulB⁺ allele from F'104/sulB diploid*[a]

Donor	F⁻ *leu sulB lon* recipients transduced to Leu⁺		
	Total no.	No. of MMS^s[b]	% MMS^s
Δ*lon sul*⁺	10	5	50
F'104/Δ*lon sulA*	11	5	45
F'104/Δ*lon sulB*	31	6	19
Δ*lon sulB leu*⁺	23	0	<4

[a] JB3 (F⁻ *leuA sulB lon*) was obtained from J. Walker. P1CMclr100 was grown on the donor strains (SG13009, SG13402, SG13404, and SG13083) and used to transduce JB3 to Leu⁺. Transductants were purified and tested for growth on MMS plates.

[b] MMS^s, MMS-sensitive.

mine starvation, but that at later times, a second, less efficient filamentation system is activated.

Our experiments suggest some roles that SulA and SulB products may play in this *sul*-dependent filamentation. Since *sulA* has no detectable phenotype in the absence of *lon* and the *lon sulA* double mutation has a phenotype indistinguishable from that of a *lon*⁺ strain, we would predict that all *lon* effects on *sul*-dependent filamentation are mediated through *sulA*, possibly through degradation of the *sulA* product, as suggested by George et al. (6). In addition, since insertion mutations in *sulA* can be isolated (10; S. Gottesman, unpublished data), it seems unlikely that *sulA* plays a normal, required role in cell division. Huisman and D'Ari (10) have recently reported that the *sulA* operon is UV inducible and that the level of expression is not affected by *lon*; both observations are consistent with the model suggested by George et al. and with our data.

sulB mutations, however, are relatively rare (about 10-fold less frequent than *sulA* mutations), are dominant to the wild type, and are somewhat defective for cell division, even in the absence of a *lon* mutation or an SOS-inducing treatment such as UV. In addition, the characteristic phenotype of *sulB* is found in both *lon* and *sulA* strains. No insertion mutations in *sulB* have yet been isolated.

The epistatic nature of the *sulB* mutations can be explained formally as a resistance to the *sul*-dependent filamentation of *lon sulA*⁺ strains. This result rules out models in which *sulB* acts on *sulA*, which then inhibits septation; if so, *lon sulA sulB* mutations should act like *sulA lon* rather than like *sulB*.

Dominance of *sulB* mutations could be due to mutation at a site affecting synthesis of *sulB* product or to the interaction of the *sulB* product either with itself or with some other component of the cell. The requirement for either a site mutation or a specific change in interaction of the *sulB* product with something else would explain the relative rarity of these mutations compared with the recessive *sulA* mutations. One model which incorporates the epistatic nature of the *sulB* mutations, their dominance, and their growth problems in low-salt medium postulates that the *sulB* product is a part of the cell division apparatus and a target for *sulA* action. The dominant *sulB* mutation would produce a *sulB* product resistant to the *sulA* product; as a result, the cell would divide regardless of the presence of either the *sulA*⁺ product or the inhibitable *sulB*⁺ product in the cell. The *sulA* product would be the inducible division inhibitor in such a model, consistent with the finding of UV induction of this operon (10). The *sulA* product may act to inhibit cell division by interacting with and thus blocking the action of the *sulB* product. The tendency of *sulB* strains to filament in low-salt medium may reflect the change necessary in this essential product to make it resistant to *sulA* but less fit for its normal function.

Relationship between *sfi* and *sul* mutations. Similar studies of the physiology of *sfiA* and *sfiB* mutations have recently been carried out by Huisman et al. (12). They have confirmed that *sfiA* mutations map similarly to *sulA* mutations. Although many of their *sfiA* alleles are recessive, as were our *sulA* mutations, some were dominant. We have not detected a dominant *sulA* mutation in the five *sulA* alleles we have tested.

The *sfiB* mutations described by Huisman et al. (*sfiB114* and *sfiB103*) are also recessive, whereas our *sulB* mutations (six were examined) are all dominant. A number of differences exist between the two sets of experiments: Huisman et al. (12) assayed UV sensitivity and temperature resistance in a *tif lon* strain, whereas we assayed filamentation and MMS sensitivity in a *tif*⁺ *lon* strain. We introduced the *tif* allele into our *lon sul*⁺ background and compared the behavior of this *tif lon sul*B⁺ strain with that of an isogenic *tif lon sulB*367 strain at high temperature. Whereas the *sulB* mutation conferred MMS resistance on the *tif lon* strain, it did not completely block the *tif*-induced, high-temperature filamentation or significantly affect viability at high temperatures. In addition, a *sfiB104* allele (not one of the *sfiB* alleles tested by Huisman et al.) introduced into our *tif*⁺ *lon* strain was dominant for MMS resistance (data not shown).

Thus, both the dominance of *sulB* and *sfiB* mutations and their effectiveness in suppressing the *tif lon* filamentation seems to be somewhat

different in our strain background from that used by Huisman et al. Since the same *sulB* and *sfiB* alleles have not been tested in the two systems, it is possible that two classes of mutations exist, one recessive and one dominant. It seems more likely to us that the differences reflect slight changes in the balance of amounts of other components of this undoubtedly complicated system in the two strain backgrounds which lead to somewhat different dominance results in the two systems. Although we do not understand the basis for the differences, the result supports the notion that *sulB* mutations are not dominant as a result of a site mutation.

ACKNOWLEDGMENTS

We thank Michael Gottesman, Max Gottesman, and Sankar Adhya for their comments on the manuscript and Mark Willingham for his help with the microscopy experiments.

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