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Second-Site Mutations in *capR(lon)* Strains of *Escherichia coli* K-12 That Prevent Radiation Sensitivity and Allow Bacteriophage Lambda to Lysogenize

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capR(lon) mutants of *Escherichia coli* K-12 are mucoid and sensitive to ultraviolet (UV) and X-ray radiation as well as to nitrofurantoin. The mutants form filaments after exposure to these agents. *capR* mutants are also conditionally lethal since they die when plated on complex medium even without UV treatment; this phenomenon is designated "complex medium-induced killing." Furthermore, *capR* mutants are poorly lysogenized by bacteriophage λ. Second-site revertants were isolated by plating on media containing nitrofurantoin. All 17 of the independent revertants studied were still mucoid but resistant to UV radiation. Sixteen of the 17 revertants contained a mutation, *sulA*, that cotransduced with *pyrD* (21 min). A second locus, *sulB*, was also found that cotransduced with *leu* (2 min). Studies with partial diploids (F'*pyrD*$^+$ *sulA*$^+$/*pyrD36 sulA17 capR9(lon)* demonstrated that *sulA*$^+$ is dominant to *sulA*; thus the indicated partial diploid is UV sensitive, whereas the haploid parent is UV resistant. Furthermore, two other phenotypic traits of *capR(lon)* mutants were reversed by the *sul* mutation: complex medium-induced killing and the inability of λ phage to efficiently lysogenize *capR* strains. On the basis of these and other results, the following model is suggested to explain *capR(lon)* and *sul* gene interactions. *capR(lon)* is a regulator gene for the structural genes *sulA*$^+$ and *sulB*$^+$. Derepression of both *sul* operons results in UV sensitivity and decreased ability of λ to lysogenize, whereas inactivation of either *sul*$^+$ protein by mutation to *sul* prevents these phenomena.

Ten enzymes involved in capsular polysaccharide synthesis in *Escherichia coli* K-12 are controlled by a single gene called *capR(lon)* (12; A. Markovitz, *In* I. Sutherland [ed.], *Surface Carbohydrates of Procaryotic Cells*, in press). The *capR*-specified protein appears to be a repressor, though it has not been isolated (Sutherland, ed., in press). In wild-type bacteria, *capR*$^+$ protein represses the synthesis of enzymes located in at least four spatially separate operons involved in capsular polysaccharide synthesis. In *capR* mutants, the *capR* protein is inactive and consequently no longer represses these four operons. As a result, the operons are derepressed and the enzymes are overproduced (A. Markovitz, in press). The overproduction of enzymes involved in capsular polysaccharide synthesis causes *capR* colonies to be mucoid on minimal agar plates.

Further studies with *capR* mutants show that in addition to overproducing capsular polysaccharides, *capR(lon)* strains are extremely sensitive to ultraviolet (UV) and X-ray radiation (2, 3, 11, 16, 21). After irradiation, *capR* mutant strains, but not *capR*$^+$ strains, form nonseptate filaments that die when incubated in complex media. The formation of a filament without septation indicates that the *capR* mutants are defective in cell division. Our working hypothesis is that the *capR* gene controls cell division and UV sensitivity in the same way that the *capR* gene controls polysaccharide synthesis, i.e., the *capR* protein controls an operon(s) whose structural genes are involved in UV sensitivity and cell division. A *capR* mutant will have an inactive repressor which will result in derepression of the gene(s) involved. This overproduction of the protein(s) involved in cell division and UV sensitivity is lethal to the cell after irradiation and gives rise, either directly or indirectly, to the nonseptate filaments.

If *capR* controls cell division and UV sensitivity the same way it controls polysaccharide synthesis, in a *capR* mutant background, one should be able to isolate strains that have mutated in the structural genes coding for proteins involved in UV sensitivity such that these

strains will be UV resistant. In this paper we report on the isolation of second-site mutations that conform to the above expectation. These mutations prevent UV sensitivity and filament formation in *capR* mutants but do not affect the mucoid phenotype. One set of second site mutations were co-transducible with *pyrD* (21 min). This genetic locus is similar if not identical to *sul* (suppressor of *lon*) recently mapped by Johnson and Greenberg (13). We designate this locus *sulA*. A second locus, *sulB*, was also found which was co-transducible with *leu* (2 min).

We determined that the F′ episome, F′106, contains *sulA*$^+$ gene(s). Using this episome, we demonstrated that *sulA*$^+$ is dominant to *sulA* in a *capR* mutant background. Thus, the partial diploid is UV sensitive, whereas the haploid parent is UV resistant. The fact that *sulA*$^+$ is dominant was predicted from the hypothesis and supports the model that *sulA* is a structural gene(s) coding for a protein(s) involved in expression of the UV-sensitive phenotype. We further show that two other phenotypic traits of *capR(lon)* mutants in addition to UV sensitivity are reversed by the *sul* mutations.

MATERIALS AND METHODS

Bacterial and bacteriophage strains. All strains are derivatives of *Escherichia coli* K-12 (Table 1). The bacteriophage used for generalized transduction was a mutant of P1*kc* (called P1 for brevity) isolated in this laboratory that grows on *galU* strains (strain MC169) and adsorbs to *E. coli* more efficiently that P1*kc*. It is probably similar to the mutant of P1*kc* (designated P1*clG*) first described by Franklin (7). Bacteriophage λ (wild type), λ*susN*7, λ*cI*, λ*vir*, λ*cI857susS*7 and amber mutants in bacteriophage T4 (T4*am*7, *am*13, *am*33, *am*37, *am*55) were also used. The λ mutants were provided by A. Campbell, and the T4 mutants were provided by R. Haselkorn.

Media. Complex medium was YET (5 g of yeast extract, 10 g of tryptone, 10 g of NaCl, and water to 1 liter [15]) and the minimal medium was M9 (1) supplemented with 0.6% glucose, thiamine, amino acids, and adenine as required. These media were solidified by adding either 1.5%, or for experiments with λ, 2.2% agar (23). YET broth supplemented with 2.5 mM $CaCl_2$ was used for transduction with P1.

TABLE 1. *Strains of Escherichia coli K-12*

Strain	Sex	Relevant genotype	Derivation, source, and/or genotype
MC100	F$^-$	*sul*$^+$	R. Curtiss III (his strain χ-156); *leu-6 proC34 purE42 trypE38 thi-1 ara-14 lacY1 galK2 xyl-5 mtl-1 tonA23 tsx-67 azi-6 str-109* λ$^-$ *pon*$^-$ *capR*$^+$ *sul*$^+$ *supE44*
MC169	F$^-$	*proC*$^+$ *capR9(lon) galU*	A *proC*$^+$ *capR9 trp*$^+$ *galU* derivative of MC100 obtained by two consecutive transductions (12); the *capR9* allele was originally isolated on an F′13 episome
RGC101	F$^-$	*capR*$^+$ *supE44*	P1 (MC169) × MC100; selection for Pro$^+$; score nonmucoid
RGC103	F$^-$	*capR9(lon) sul*$^+$	P1 (MC169) × MC100; selection for Pro$^+$; score *capR9* as a mucoid, UV-sensitive clone
X7102	F$^-$	*capR*$^+$ *proC*	J. Beckwith via D. Court; *proC trp lac*ΔX74 *str*; a strain containing no known suppressors
RGC121	F$^-$	*capR*$^+$	P1 (MC169) × X7102; selection for Pro$^+$; score nonmucoid
RGC123	F$^-$	*capR9(lon) sul*$^+$	P1 (MC169) × X7102; selection for Pro$^+$; score *capR9* as a mucoid, UV-sensitive clone
W620	F$^-$	*pyrD36*	*E. coli* Genetic Stock Center no. 4278, Paris strain; *thi-1 pyrD36 gltA6 galK30 str-129 rel-1?* λ$^-$ *supE44?*
NS31	HfrH	*proC*	Norman Schwartz; derivative of strain AB259
LYC100	F$^-$	*proC pyrD36*	NS31 × W620; selection for Gal$^+$ Glt$^+$; score *proC pyrD36*
LYC101	F$^-$	*pyrD36 sul*$^+$ *capR9*	P1 (MC169) × LYC100; selection for Pro$^+$; score *capR9* as a mucoid, UV-sensitive clone
LYC102	F$^-$	*pyrD36 sulA17 capR9*	Spontaneous nitrofurantoin and UV-resistant mutant of LYC101 isolated from a nitrofurantoin plate; retains mucoid phenotype
KLF6/KL181	F′	F′*pyrD*$^+$, *sul*$^+$/*pyrD34*	*E. coli* Genetic Stock Center no. 4309; *thi-1 pyrD34 his-68 trp-45 recA1 galK35 mtl-2 xyl-7 malA1 str-118* λ^R, λ$^-$
LYC103	F′	F′*pyrD*$^+$ *sul*$^+$/*pyrD36 sulA17 capR9*	KLF6/KL181 × LYC102; selection for *pyrD*$^+$
KLF25/KL181	F′	F′*trp*$^+$ *pyrD*$^+$ *sul*$^+$/*pyrD34*	*E. coli* Genetic Stock Center no. 4320; *thi-1 pyrD34 his-68 trp-45 recA1 mtl-2 xyl-7 malA1 galK35 str-118* λ^R, λ$^-$
AB259	HfrH	*purE*$^+$ *capR*$^+$ *sulB*$^+$	E. Adelberg; HfrH
RGC130	F$^-$	*capR*$^+$ *proC34*	AB259 × MC100; selection for Pur$^+$ and retention of Pro$^-$; score for loss of *supE44* by inability to yield plaques with either T4*am*55 or T4*am*33 phage
RGC131	F$^-$	*capR*$^+$	P1 (MC169) × RGC130; selection for Pro$^+$; nonmucoid
RGC133	F$^-$	*capR9*	P1 (MC169) × RGC130; selection for Pro$^+$; score *capR9* as a mucoid, UV-sensitive clone
HC1002	F$^-$	*capR9*	Reference 12

Isolation of *sul* mutants. *sul* mutants were isolated by plating 10^4 to 10^6 exponentially growing *capR9(lon) sul*$^+$ bacteria on YET agar containing 2 μg of nitrofurantoin per ml (Sigma) where nitrofurantoin is indicated. The drug mimics UV treatment in that it induces filament formation and death in *capR(lon)* strains (14) as it does in *E. coli* B (18). *capR9 sul* survivors (one survivor from approximately 10^4 cells plated) appear as single clones under these conditions and were purified by streaking on YET containing NF.

Genetic procedures. Conjugation experiments were performed by mixing exponentially growing donors and recipients (1:10) in YET broth. After 90 min at 37°C the mating mixtures were agitated vigorously on a Vortex mixer for 30 s before plating. Transduction experiments followed the procedure of Miller (17) using bacteriophage P1 at multiplicity of infection between 0.1 and 1. The P1 phage used for the transductions were grown twice on the donor using the soft agar overlay technique (17).

Mucoid and radiation sensitivity testing. Isolated *pyrD*$^+$ uracil-independent clones from transduction plates were patched directly on minimal medium without uracil and grown overnight at 37°C. These patches, used for testing UV sensitivity (*sul* or *sul*$^+$) as described below, were not purified by streaking. However, similar co-transduction frequencies for *pyrD* and *sul* were obtained with *pyrD*$^+$ transductants purified by streaking before testing their response to UV by the patch test (footnotes to Tables 2 and 3). The patch test on minimal medium reveals the mucoid state of *capR9(lon) sul*$^+$ or *capR9(lon) sul* bacteria and the nonmucoid state of *capR*$^+$*(lon*$^+$*)* strains. The patch plate was replicated onto a YET agar plate and incubated for 2 to 5 h at 37°C. This YET plate was then used to replicate another YET plate which was then treated with UV for 60 s at a distance of 20 inches (ca. 50.8 cm) from two germicidal lamps. A second minimal agar plate was also replicated as a growth control. Under these conditions, after overnight growth at 37°C the *capR9 sul*$^+$ strain does not grow (UV sensitive), whereas either a *capR9 sul* strain or a *capR*$^+$ strain grows confluently (UV resistant). UV-resistant and UV-sensitive strains were always used as controls in the patch tests. These results correlate well with previous quantitative killing curves which show that a *capR*$^+$*(lon*$^+$*)* strain is UV resistant, whereas a *capR9(lon) sul*$^+$ strain is UV sensitive (15). In a similar quantitative test with a *capR9 sulA1* strain (15) UV resistance was apparent (42% survival after a 30-s UV dose that kills 99.9% of a *capR9 sul*$^+$ strain).

Curing of F′ strains. Curing of F′ strains with acridine orange was carried out essentially as described by Hirota (10).

Complex medium-induced killing. Bacteria were grown to either stationary phase or exponential phase in minimal medium. Cells were diluted in phosphate-buffered saline containing gelatin (4) and then plated on YET and minimal agar. The percent survival is calculated as follows: colony count on YET divided by the colony count on minimal medium multiplied by 100.

Sucrose velocity sedimentation. Ten milliliters of exponentially grown culture were washed with 0.01 M tris(hydroxymethyl)aminomethane (Sigma)-hydrochloride buffer (pH 7.4) and resuspended in 0.5 ml. The cell suspension was layered on a 5 to 20% sucrose gradient. The gradients were centrifuged in the SW41 rotor of the Spinco model L3-50 centrifuge for 4 min at an average speed of 3,000 rpm. Gradients were collected by puncturing the bottom of the tubes and collecting 12-drop fractions (approximately 0.2 ml). Each fraction was collected in tubes with 0.8 ml of cold 0.85% NaCl. Optical density at 600 nm was read on a Gilford 240 spectrophotometer. The radioactivity was determined on a 100-μl sample of each fraction which was added to chilled 5% trichloroacetic acid and filtered on GF/C glass fiber filter. The filters were then washed twice with 5% trichloroacetic acid and once with 70% ethanol-3% potassium acetate, dried, and counted in a liquid-scintillation counter as described (12).

Procedures with bacteriophage. Growth of T4 amber and λ amber mutants was measured by spot tests in which 10^4, 10^3, and 10^2 phage particles were spotted on appropriate bacterial lawns.

λ lysogeny was measured as described by Echols et al. (6) with the following modifications. Bacteria were grown to stationary phase in M9 glucose, diluted 1:25 into YET + 0.2% maltose, and grown for approximately 2.5 generations. Immunity was determined using λ*cl* and λ*vir* on YET plates containing 2.2% agar. The *capR9 sul*$^+$ strain was spread on both minimal and YET plates to determine the number of cells per milliliter and the percent survival after phage infection. This procedure was adopted to determine whether there is an effect of complex media-induced killing on lysogenization by λ in the *capR9 sul*$^+$ strain.

RESULTS

Isolation and transduction mapping of *sul* mutants. *sul* mutants were isolated in a *capR9 supE44* (strain RGC103) and a *capR9* suppressor free background (strain RGC123) (see Materials and Methods; Table 1). All of the *capR9 sul* mutants were mucoid at 37°C on minimal agar as are the *capR9 sul*$^+$ parent strains; however, the *capR9 sul* mutants are resistant to UV irradiation.

All of the *sul* mutants isolated in strains RGC103 and RGC123 were tested for the presence of amber suppressors as a possible cause of the UV-resistant phenotype using λ and T4 mutants. No evidence for increased growth of either λ or T4 amber mutants was detected in *capR9 sul* mutants (see Materials and Methods).

Preliminary conjugation experiments indicated that several *sul* mutants mapped in the *gal-trp* region. After the recent experiments of Johnson and Greenberg (13), a *pyrD36 capR9 sul*$^+$ strain was constructed (LYC101) and used as a recipient in transduction experiments to determine whether our series of independently isolated mutations could be co-transduced with

pyrD$^+$. Sixteen out of 17 independent *sul* mutations were co-transducible with *pyrD* (Table 2). The *sul* locus linked to *pyrD* is designated *sulA*, and the one not linked to *pyrD* (in strain RGC103-9) is designated *sulB*.

Conjugation experiments with *sulB* strain RGC103-9 located *sulB* near *leu*. This was confirmed by co-transduction of *sulB*$^+$ with *leu*$^+$ as follows. P1 grown on strain AB259, a *leu*$^+$ (presumably) *sulB*$^+$ strain, was used to transduce *leu*$^+$ into the *capR9 leu sulB* strain RGC103-9. Thirty-six *leu*$^+$ clones were purified by restreaking on selective medium. Seventeen out of 36 *leu*$^+$ transductants (47%) had acquired the *sulB*$^+$ phenotype and were thus UV sensitive.

Partial diploid analysis of *sulA*. The transduction results obtained using the strain containing episome F'106 (KLF6/KL181) are important to this study. Strain KLF6/KL181 was known to contain *pyrD*$^+$ on the F' and *pyrD34* and *recA1* on the chromosome. Thus, P1 grown on strain KLF6/KL181 should contain *pyrD*$^+$ only from the F'. We verified the presence of a chromosomal *pyrD* mutation by obtaining a *pyrD* clone after growth of the strain in acridine orange. The results of transduction studies (Table 3) indicate that the F'106 episome contains *sulA*$^+$ since 41% of the *pyrD*$^+$ transductants were *sulA*$^+$ when a *pyrD36 sulA17* strain (LYC102) was used as a recipient. A second larger F' episome in strain KLF25/KL191 also contained *sulA*$^+$ (Table 3). We note that the frequency of co-transduction is lower (41 to 47%) where the donor was *sulA*$^+$ and the recipient *sulA17* as compared with the approximately reciprocal transductions where the donor was any *sulA* (63–81%, Table 2) and the recipient was *sulA*$^+$. The difference does not appear to be related to the proximity of the sex factor to the *sulA*$^+$ locus (20) since an F$^-$*sulA*$^+$ donor yielded the same co-transduction frequency as the F'*sul*$^+$ donors (Table 3). Furthermore, the differences in the approximately reciprocal *pyrD*$^+$*sul*$^\pm$ co-transduction frequencies were reduced to within 8% of one another when single clone isolations were performed before testing (Table 2, footnote *b*; Table 3, footnote *a*).

The F' strain KLF6/KL181 was conjugated with recipient strain LYC102 (*capR9 pyrD36 sulA17*), and *pyrD*$^+$ sexductants were analyzed.

TABLE 2. *Co-transductional mapping of pyrD and sul*[a]

P1 donor	Recipient	No. of transductants tested	No. of *sul* clones	% *sul*
RGC103-1 (*sul-1*)	LYC101 (*sul*$^+$)	241	159	66
RGC103-2 (*sul-2*)	LYC101 (*sul*$^+$)	182	133	73[b]
RGC103-3 (*sul-3*)	LYC101 (*sul*$^+$)	186	118	63
RGC103-4 (*sul-4*)	LYC101 (*sul*$^+$)	195	149	76
RGC103-5 (*sul-5*)	LYC101 (*sul*$^+$)	46	33	72
RGC103-6 (*sul-6*)	LYC101 (*sul*$^+$)	121	93	77
RGC103-7 (*sul-7*)	LYC101 (*sul*$^+$)	108	71	66
RGC103-8 (*sul-8*)	LYC101 (*sul*$^+$)	137	90	66
RGC103-9 (*sul-9*)	LYC101 (*sul*$^+$)	236	0	0
RGC103-10 (*sul-10*)	LYC101 (*sul*$^+$)	129	90	70
RGC123-1 (*sul-11*)	LYC101 (*sul*$^+$)	47	30	64
RGC123-2 (*sul-12*)	LYC101 (*sul*$^+$)	177	127	72
RGC123-3 (*sul-13*)	LYC101 (*sul*$^+$)	186	137	74
RGC123-4 (*sul-14*)	LYC101 (*sul*$^+$)	206	145	70
RGC123-5 (*sul-15*)	LYC101 (*sul*$^+$)	137	111	81
HC1002-1 (*sul-16*)	LYC101 (*sul*$^+$)	196	142	72

[a] All recipients were *capR9, pyrD36* and the selected allele in every case was *pyrD*$^+$. A control transduction with strain RGC103 (*sul*$^+$) as donor and strain LYC101 (*sul*$^+$) as recipient yielded no *sul* transductants out of 162 *pyrD*$^+$ transductants.

[b] When 47 transductant clones were first purified by streaking and then tested for *sul* (see Materials and Methods) the co-transduction frequency was 10% lower than indicated in the table.

TABLE 3. *Co-transduction mapping of pyrD and sul*$^+$ *in episome-containing strains*

P1 donor	Recipient	No. of transductants tested	No. of *sul*$^+$ clones	% *sul*$^+$
KLF6 (*sul*$^+$)/KL181	LYC102 (*sul-17*)	182	75	41
KLF25 (*sul*$^+$)/KL191	LYC102 (*sul-17*)	120	57	47
RGC103 (*sul*$^+$)	LYC102 (*sul-17*)	112	53	47[a]

[a] When 49 transductant clones were first purified by streaking and then tested for *sul* (see Materials and Methods) the co-transduction frequency was 8% higher than indicated in the table.

Thirty-six out of 67 *pyrD*$^+$ clones (54%) initially obtained were sensitive to UV as well as to nitrofurantoin. Twelve of the presumptive partial diploid clones that were UV sensitive were grown in varying concentrations of acridine orange to determine whether the F′ episome could be cured. After growth in acridine orange (see Materials and Methods) 48 single clones were isolated on nonselective medium from each of the 12 original clones. Four out of the 12 strains yielded 48 clones which were *pyrD*, UV resistant, and mucoid (*capR9 sulA17*). One of the 12 yielded 45 *pyrD* clones that were UV resistant and mucoid and 3 *pyrD*$^+$ clones that were UV resistant and mucoid. Thus, 5 out of 12 clones were demonstrated to be partial diploids indicating that *sulA*$^+$ (UV sensitivity) is dominant to *sulA17* (UV resistance) in a *capR9* background. The other 7 clones (out of 12) remained *pyrD*$^+$, mucoid and UV sensitive. These may have been haploid recombinants (*pyrD*$^+$ *capR9 sul*$^+$) or stable partial diploids.

Complex medium-induced killing. It was first observed by Adler and Hardigee (2) that unirradiated *capR*(*lon*) cells grown in complex broth consist of a mixture of short, normal appearing cells and nonseptate filaments. We confirm the existence of filaments in such bacteria. In one particular *capR9* strain, RGC123, the presence of filaments in complex broth was very evident upon microscopic observation (Fig. 1b). Furthermore, filaments in the *capR9 sul*$^+$ (RGC123) cell population appear to be induced upon transfer from minimal to complex medium (Fig. 1a and b). In contrast, the isogenic *capR*$^+$ strain, RGC121, produces uniform rods in complex medium (Fig. 1c). This heterogeneity of cell sizes was also demonstrated by centrifuging the cells in a velocity sucrose gradient (Fig. 2). The *capR9* cells are present throughout the sucrose gradient (Fig. 2a), whereas the *capR*$^+$ cells sediment as a discrete band near the top of the sucrose gradient at a position characteristic of the normal bacterial cells (Fig. 2b). The *capR9* filamenting cells as well as the normal cells incorporate [^{3}H]leucine

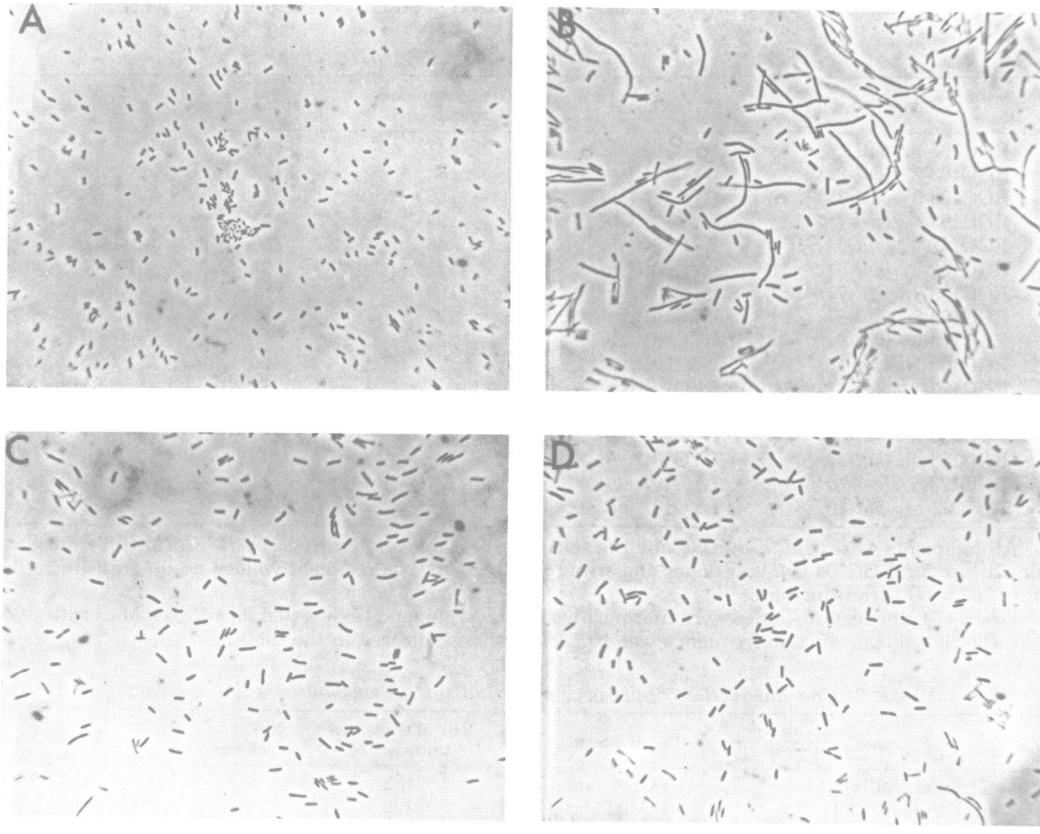

FIG. 1. *Bacterial morphology in minimal and complex media. (A) RGC123 (capR9) in minimal medium; (B) RGC123 subcultured in YET broth; (C) RGC121 (capR*$^+$*) subcultured in YET broth; (D) RGC123-2 (capR9 sulA12) subcultured in YET broth.*

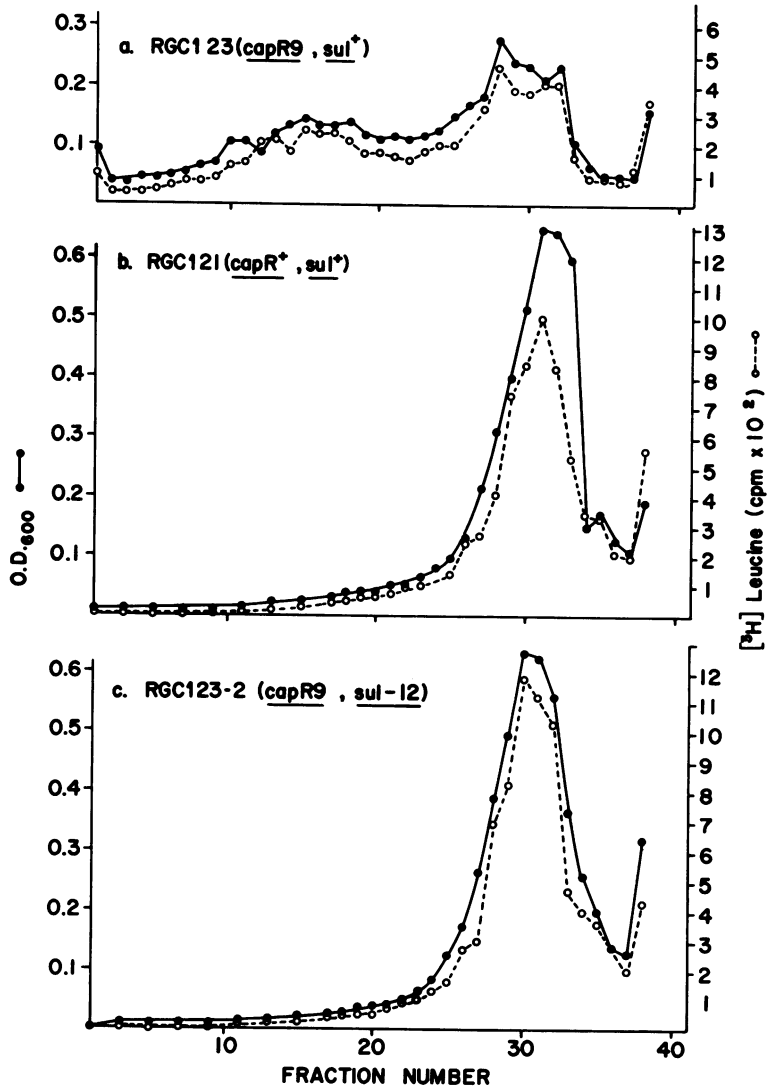

FIG. 2. *Sucrose velocity sedimentation profiles of whole cells from cultures grown in YET broth. (a) RBC123 (capR9 sul+); (b) RGC121 (capR+ sul+); (c) RGC123-2 (capR9 sul-12).*

into protein in general agreement with qualitative results previously published (24). The presence of these filaments in complex medium suggests that some type of abnormal cell division is occurring without UV irradiation. The defect is revealed in the *capR9* strain, RGC123, by its longer doubling time in YET broth at 37°C (42 min) compared to the isogenic *capR*+ derivative (30 min); and by the lower viable cell titer of the *capR9* strain, determined by dilution plating on YET agar, compared to YET broth cultures of the isogenic *capR*+ derivative (vide infra). In contrast, when *capR9* cells are grown in liquid minimal media, a growth condition in which no filaments are observed (Fig. 1a), there is no difference between *capR*+ and *capR9* strains in doubling time (80 min) and in the viable cell count of minimal cultures at similar turbidity. Furthermore, the viable cells per milliliter in exponential minimal medium cultures of both *capR9* and *capR*+ strains, as determined on minimal agar, are approximately 70% of the particle count (Petroff-Hausser counting chamber).

Using the above observations, an assay to determine the fraction of cells that die in complex medium (YET) was designed. Since the *capR9* strain only survives completely in minimal medium, the *capR9* mutant cells were grown in minimal medium, diluted, and then

spread on YET plates and minimal plates. The percentage of survival is defined as the clones on YET plates divided by the clones on minimal plates multiplied by 100.

The greatest complex medium-induced killing is observed with strain RGC123 in which only 3% of a stationary phase (overnight) and 12% of an exponential phase culture form clones on YET agar (Table 4). In another *capR9* strain, RGC103, the percent survival on YET are 21 and 27, respectively (Table 4). Complex medium-induced killing can be observed when the cells were grown in YET broth and then plated on YET agar and minimal agar plates, but the results were more variable.

Since UV sensitivity and polysaccharide production can be suppressed by specific nonsense suppressor mutations (15) and strain RGC103 contains *supE44*, a suppressor-free derivative of strain RGC103 was contructed. Comparison of strain RGC103 and the suppressor-free derivative, RGC133, (Table 4) indicates that removal of this suppressor does not increase the complex medium-induced killing.

Complex medium-induced killing of the *capR9* bacteria is not mediated by visible light, since experiments done under conditions that prevent photoreactivation (i.e., darkness) do not prevent killing.

The conditions that rescue filaments after UV irradiation also rescue complex medium-induced filaments. That is, when strain RGC123 was incubated on YET plates containing 0.1 M pantoyl lactone (3) or spread onto YET plates which were incubated under anaerobic conditions, no complex medium killing was observed.

Effect of *sul* on complex medium-induced killing. Introduction of either *sulA* or *sulB* mutations into the *capR9* strains prevent both spontaneous filament formation and complex medium-induced killing. The *capR9 sulA12* strain (RGC123-2) produced only short rods in complex medium (Fig. 1d), and centrifugation of the cells in a velocity sucrose gradient results in a discrete band near the top identical to the *capR*$^+$ strain (Fig. 2c). The results presented in Table 4 show that either a *sulA* or *sulB* mutation prevents the loss of viability due to growth in complex medium in *capR9* strains. In fact, the *capR9 sul* strains constantly yield more colonies on YET agar than on minimal agar (Table 4). Thus, the *sul* mutations not only make *capR9* cells more UV resistant but also prevent the lethal filamentation caused by complex medium.

Effect of *sul* on lysogeny by bacteriophage λ. Walker et al. (23) showed that λ forms clear plaques on *capR* (*lon*) strains, although it forms turbid plaques (lysogeny) on *capR*$^+$(*lon*$^+$) strains. We have investigated this finding in *capR9*(*lon*)*sul*$^+$ and *capR9*(*lon*)*sul* strains. We have confirmed the results of Walker et al. (23) demonstrating that λ forms clear plaques on a *capR9 sul*$^+$ strain. In contrast, λ yielded turbid plaques on a *capR9 sulA12* strain (RGC123-2) as well as on a *capR9 sulA2* strain (RGC103-2) and a *capR9 sulB* strain (RGC103-9). The fact that λ yields turbid plaques in either a *capR9 sulA* or *capR9 sulB* strain suggested that the mutation of *sul*$^+$ to *sul* restores the ability of the strain to be lysogenized. The quantitative measurement of lysogenization frequency (Table 5) establishes that the *sulA12* mutation does indeed restore the capacity of the *capR9* strain to be lysogenized. Thus, a *sul* mutation in a *capR* strain allows λ to lysogenize at normal efficiency.

The effect of complex medium-induced killing on the lysogenization frequency in the *capR9 sul*$^+$ strain was neglible, since the percentage of lysogenization did not change signif-

TABLE 4. *Complex medium-induced killing*

Strain	Genotype	% Survival[a]	
		Stationary culture	Exponential culture
RGC121	*capR*$^+$	95 ± 4	90 ± 13
RGC123	*capR9*	3.2 ± 3.6	12 ± 5
RGC101	*capR*$^+$ *supE44*	89 ± 12	116 ± 16
RGC103	*capR9 supE44*	21 ± 5	27 ± 15
RGC131	*capR*$^+$	106 ± 14	88 ± 12
RGC133	*capR9*	13 ± 7	31 ± 3
RGC123-2	*capR9 sulA12*	124 ± 27	118 ± 9
RGC103-2	*capR9 sulA2*	118 ± 17	123 ± 24
RGC103-9	*capR9 sulB1*	116 ± 22	115 ± 24

[a] Results are the average of three or more separate experiments. Percent survival is calculated as follows: colony count on YET/colony count on minimal medium × 100.

TABLE 5. *Effect of sulA on the establishment of lysogeny by bacteriophage* λ[a]

Host strain	Survivors (%)[b]	Lysogenization (%)[b]
RGC121 (*capR*$^+$ *sul*$^+$)[c]	77	17
RGC123 (*capR9 sul*$^+$)[c]	47	4
RGC123 (*capR9 sul*$^+$)[d]	70	2
RGC123-2 (*capR9 sulA12*)[c]	67	27

[a] The bacterial cells were infected at a multiplicity between three and four phage per bacterium.

[b] The frequency of survival and lysogeny were determined from colony counts and immunity test as described in Materials and Methods. The lysogenization percent refers to the fraction of surviving cells which become lysogenic.

[c] Bacteria were spread on YET plates after λ infection.

[d] Bacteria were spread on M9 minimal plates after λ infection.

icantly when the *capR9 sul*$^+$ cells were spread on either YET agar plates or minimal agar plates (Table 5).

DISCUSSION

By analogy with capsular polysaccharide regulation, we hypothesized that UV sensitivity and filament formation in *capR* mutant bacteria are due to the inactivation of the *capR* repressor. Thus, our model for increased sensitivity to UV irradiation of *capR* mutants suggests that some enzymes involved in cell division and/or deoxyribonucleic acid (DNA) repair are derepressed. This hypothesis is consistent with the results of our present study. Second-site mutations were isolated that prevent radiation sensitivity but not the overproduction of capsular polysaccharide. Sixteen out of 17 independent *sul* mutations isolated in two different genetic backgrounds were co-transducible with *pyrD* (21 min). We designate this locus *sulA* since it is probably the same genetic locus recently mapped by Johnson and Greenberg (13). They reported co-transduction frequency of *sul* in one direction, where the donor was *sul*$^+$ and the recipient *pyrD36* to be 31% (12). Our co-transduction frequencies showed linkage between *sulA* and *pyrD36* to be 41 to 47% when the donor was *sul*$^+$ and 63 to 81% when the donors were *sul* mutants (Tables 3 and 4). One *sul* mutant was not co-transducible with *pyrD* and thus represented a second locus, *sulB* which was shown to be 47% co-transducible with *leu* (2 min). These mutations, *sulA* and *sulB*, conform to the hypothesis proposed. They prevent UV sensitivity and filament formation in *capR* mutant bacteria but do not eliminate the mucoid phenotype.

The mutations *sfiA* and *sfiB* (*sfi* = suppressor of filament induction) recently isolated and mapped by George et al. (8) may be identical to the mutations, *sulA* and *sulB*. The *sfi* mutants were isolated as spontaneous thermoresistant revertants of a *tif lon* strain. The *tif lon* strain forms filaments and dies at 41°C, unless secondary mutations are acquired that make the *tif lon* cells either more UV sensitive (*recA, lex, zab*) or less UV sensitive (*sfiA* and *sfiB*). *sfiA* was 53% co-transducible with *pyrD* and *sfiB* was 25 to 32% co-transducible with *leu*$^+$ (8).

Our hypothesis is further strengthened by the finding that *sulA*$^+$ is dominant to *sulA* in a *capR* mutant. This was demonstrated using an F′ episome of strain KLF6/KL181 which contains *sulA*$^+$ in addition to *pyrD*$^+$. This F′ episome, when introduced into a *sulA17 capR9* strain, caused the diploid (F′*sulA*$^+$/*sulA17 capR9*) to be UV and nitrofurantoin sensitive. Thus, the *sulA* mutation, which prevents UV sensitivity in the *capR9* strain, probably results in inactivation of a structural gene product.

The discovery that the *capR9*(*lon*) strains exhibit complex medium-induced killing (conditional lethality) in the absence of UV treatment is a significant finding. It establishes that the *capR9* mutation causes a defect that can be recognized and studied without introducing external damage to DNA.

The protein product of *sul*$^+$ has not been identified and may provide one of the keys to our understanding of cell division and radiation sensitivity. What is the protein product of *sul*$^+$? There are two important phenotypes of unirradiated *capR9 sul*$^+$ strains that are reversed by the *sul* mutations and which must be explained by any model for *capR sul* interaction. (i) *capR9 sul*$^+$ strains are conditionally lethal when plated on complex medium, whereas the *capR9 sul* mutants are not. Therefore, there is a defect in *capR sul*$^+$ strains (in the absence of UV treatment) similar to that revealed by UV treatment, and this defect can be reversed by the *sul* mutation. A model already proposed by Witkin (25) to explain UV sensitivity of *E. coli* B (a *lon* mutant [5]) invokes DNA damage as a requirement for inactivation of a repessor that controls cell division. Such a hypothesis may be retained if one postulates the damage exists in unirradiated *lon*(*capR9*) cells. (ii) λ yields clear plaques on a *capR9 sul*$^+$ strain and turbid plaques on a *capR9 sul* strain. Sussman and Ben-Zeev (20) have hypothesized that the inducer of λ repressor is damaged DNA. They demonstrated that purified λ repressor binds to DNA with single-strand breaks. Such breaks can be produced in whole cells by a number of treatments including UV. In addition, West et al. (24) reported that γ-ray-induced, single-strand breaks lead to immediate λ repressor inactivation in λ lysogens in the absence of de novo protein synthesis. Increased spontaneous λ prophage induction has been reported in both DNA ligase-deficient bacteria (9) as well as in *capR*(*lon*) mutants (23). If we propose that the *sul*$^+$ product is a nuclease then we can explain the *capR* and *sul* genes interaction. Overproduction of *sul*$^+$ product in a *capR9 sul*$^+$ strain could provide more nicked DNA as a substrate for λ repressor binding, thus preventing the increase in free λ repressor concentration that is required to establish lysogeny. A *sul* mutation in a *capR9* strain would inactivate the *sul* nuclease and lysogeny would again be readily established. Increased nuclease activity in a *capR9 sul*$^+$ strain could also explain both le-

thality in complex medium and increased sensitivity to UV.

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