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Fine structure mapping and properties of mutations suppressing the *lon* mutation in *Escherichia coli* K-12 and B strains

By BEN F. JOHNSON
Palo Alto Medical Research Foundation, Palo Alto, California

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SUMMARY

Mutations, *sulA* and *sulB*, that suppress the UV sensitivity conferred by the *lon* mutation have been isolated and precisely positioned on the linkage map of *Escherichia coli*. The *E. coli* B strains Bs-3 and Bs-8 have been shown to possess *sulA* mutations. Also the *E. coli* K12 strain J6271 that possesses a suppressor of the *lon* mutation, previously designated as *suf*, has been shown to be a *sulA* mutation. A series of methylmethane sulphonate resistant derivatives of an *E. coli* K12 *lon* strain has been isolated and genetically characterized. In addition to *sulA* mutations, a second suppressor *sulB* was identified and located between *leu* and *azi* genes on the chromosome. Neither *sulA* or *sulB* mutations result in increased sensitivity to the antibiotics ampicillin, rifampicin, or actinomycin D, nor do they have any significant effect upon the overproduction of mucopolysaccharide caused by the *lon* mutation. Under some growth conditions the *sulB* mutation causes cells to be temperature sensitive for the cell division process at 42 °C.

1. INTRODUCTION

A mutation in the *lon* gene results in the inhibition of septum formation following the exposure of cells to agents that inhibit DNA replication (Howard-Flanders *et al.* 1964; Green *et al.* 1969). This inhibition can be induced by a number of agents, either by damaging the DNA structure (e.g. ultraviolet irradiation or methylmethane sulphonate) or by inhibiting steps in DNA metabolism (e.g. nalidixic acid and nitrofurans), to effect an unbalanced growth condition where the ratio of cytoplasmic mass to DNA increases in excess of the normal value (Berg *et al.* 1976; Kantor & Deering, 1968; Walker & Pardee, 1968). Derivatives of *lon* strains can be readily isolated that are as resistant to the above agents as *lon*$^+$ wild type strains (Witkin, 1946). In a previous communication the chromosomal location of a suppressor specific for the *lon* mutation in *E. coli* B and K12 strains was described (Johnson & Greenberg, 1975). The location of this *sul* gene on the chromosome was shown to be highly linked to the *fabA* gene and more distantly linked to the *pyrD* gene. A single methylmethane sulphonate (MMS) resistant strain of *E. coli* K12 and four ultraviolet (UV) resistant strains of *E. coli* B (B/r) were shown to possess a *fabA*-linked *sul* mutation.

Table 1. Escherichia coli *strains used in this study*

Designation	Genotype*	Source and comments†
	E. coli K-12 strains	
AB1157	*thi-1, thr-1, leu-6, proA2, his-4, argE3, lacY1, galK2, ara-14, xyl-5, mlt-2, strA-31, tsx-33, supE44*	E. Adelberg
D1321	*try, mal, mel, nal, str; Hfr*	I. Orskov
J62	*pro, try, his, lac, str*	Organessian
J6271	*his, lac, lon-7, sulA29*	Organessian
J67273	*try, his, lac, lon-7, str*	Organessian
J6275	*pro, his, lac, sulA29*	Organessian
KL398	*thi-1, leu-6, proC32, metE70, hisF860, thyA54, lacZ36, ara-14, mtl-1, xyl-5, malA38, str-109, spc-15*	K. B. Low
PAM 150	*thi-1, leu-6, proA2, pan, metA, lacY1, galK2, ara-14, xyl-5, mtl-1, str-31, tsx-33, supE44, non*	B. F. Johnson‡
PAM 154	*thi-1, pyrD36, lon-21, sulA23, cmlB, galK30, str-129*	B. F. Johnson‡
PAM 155	*thi-1, fabA2, lon-21, galK30, str-129*	B. F. Johnson‡
PAM 157	*thi-1, pyrD36, lon-21, galK30, cml B, str-129*	Spontaneous TC^r isolate of PAM 153‡
PAM 161	*sulB25*, other markers as PAM 660	Spontaneous isolate PAM 660 resistant to MMS
PAM 162	*sulB26*, other markers as PAM 660	Spontaneous isolate PAM 660 resistant to MMS
PAM 163	*sulA27*, other markers as PAM 660	Spontaneous isolate PAM 660 resistant to MMS
PAM 164	*sulA28*, other markers as PAM 660	Spontaneous isolate PAM 660 resistant to MMS
PAM 168	*thi-1, thr-1, proA2, his-4, metA, lon-21, sulB25, lacY1, galK2, xyl-5, mtl-2, strA-31, tsx-33, supE44*	P1 (D1321) → PAM 161, select *leu*$^+$ transductant
PAM 169	*metB1, pan, azi; Hfr*	*pan* induced by MNNG, *azi* spontaneous isolate of strain B1
PAM 170	*thi-1, lon-21, sulA24, galK30, str-129*	P1 (Bs-3) → PAM 155, select *fabA*$^+$ transductant
PAM 171	*thi-1, lon-21, sulA24, pyrD36, cmlB, galK30, str-129*	P1 (PAM 157) → PAM 170 select TC^r transductant
PAM 172	*thi-1, lon-21, sulA22, galK30, str-129*	P1 (Bs-8) → PAM 155 select *fabA*$^+$ transductant
PAM 173	*thi-1, lon-21, sulA22, pyrD36, cmlB, galK30, str-129*	P1 (PAM 157) → PAM 172 select TC^r transductant
PAM 174	*thi-1, lon-21, sulA29, galK30, str-129*	P1 (J6271) → PAM 155 select *fabA*$^+$ transductant
PAM 175	*thi-1, lon-21, sulA29, pyrD36, cmlB, galK30, str-129*	P1 (PAM 157) → PAM 174 select TC^r transductant
PAM 176	*thi-1, lon-21, sulA27, galK30, str-129*	P1 (PAM 163) → PAM 155 select *fabA*$^+$ transductant
PAM 177	*thi-1, lon-21, sulA27, pyrD36, cmlB, galK30, str-129*	P1 (PAM 157) → PAM 176 select TC^r transductant
PAM 178	*lon, pyrD36, cmlB, sulB25*	P1 (PAM 168) → PAM 160 select *leu*$^+$
PAM 660	*thi-1, thr-1, leu-6, proA2, his-4, met, lon, lacY1, galK2, ara-14, xyl-5, mtl-2, strA31, tsx-33, supE44*	J. Donch (1968)

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Table 1 (*cont.*)

Designation	Genotypes*	Source and comments†
	E. coli B strains	
B	*malB, lon*	R. Hill
Bs-3	*uvrC152 sulA24, lon*	R. Hill
Bs-8	*uvrB153, sulA22, lon*	R. Hill
PAM 158	*lon, pyrD36, cmlB*	P1 (PAM 157) → *E. coli* B select *cmlB*
PAM 159	*lon, pyrD36, cmlB, ara*	MNNG mutagenesis of PAM 158
PAM 160	*lon, pyrD36, cmlB, leu-6*	P1 (AB311) → PAM 159 select *ara*+

* The allele numbers are those assigned by *E. coli* Genetic Stock Center, Department of Microbiology, Yale University.

† Abbreviations: TC^r is tetracycline resistance, MNNG is *N*-methyl-*N'*-nitro-*N*-nitroso guanidine, MMS is methylmethane sulphonate.

‡ Johnson & Greenberg, 1975.

We have also reported data suggesting that different types of *sul* mutations may exist. A previous publication from this laboratory noted that the *E. coli* B strain Bs-8 is like B/r strains in its resistance to nalidixic acid but that strain Bs-3 is only moderately resistant to nalidixic acid (Green *et al.* 1969). Organessian & Organessian have reported a suppressor of the *lon* gene that is located in the *trp* region of the chromosome (Organessian & Organessian, 1973). In this communication we report the chromosomal location and properties of two types of suppressor mutations, *sulA* and *sulB*, present in the above strains as well as in a series of independent MMS resistant isolates of *lon* strains of *E. coli* K12 and *E. coli* B. We will compare these results with the recently reported findings of George, Castellazzi & Buttin (1975) and Gayda, Yamamoto & Markovitz (1976).

2. MATERIALS AND METHODS

(i) *Biological material and media.* The description of the bacterial strains used in this study are given in Table 1. The complete medium was LB broth and contained 10 gm tryptone, 5 gm yeast extract, and 10 gm sodium chloride/l. LBC broth was LB broth with sterile $CaCl_2$ added to a final concentration of 5×10^{-3} M. LBC broth was solidified with 0·6 % agar for a top agar in the growth of bacteriophage. The minimal medium was minimal broth Davis supplemented with glucose at 5 gm/l., L-amino acids at 100 mg/l., pantothenate and thiamine at 10 mg/l. as needed. Plates of solidified medium were prepared by adding 1·5 % Bacto-agar. MMS plates were prepared by addition of either 50 or 250 μl methylmethane sulphonate to each litre of autoclaved LB broth agar just before pouring. MMS plates were stored under refrigeration and kept no longer than five days before their use. AZ plates were prepared by adding 150 mg sterile sodium azide/l. of LB agar broth.

(ii) *Genetical methods.* Conjugations were performed according to the procedure of Freifelder (1971). Transductions were performed by the procedure of Roth

(1970). A virulent derivative of the bacteriophage P1*bc* (Chung, Greenberg & Donch, 1974) was used for this study and will be referred to in this paper as P1. Transduced cells were normally immediately spread on to the necessary selection medium subsequent to absorption of the transducing phage. In the case of the selection of *azi* transductants, transduced cells were first suspended in minimal broth Davis supplemented with glucose and the required amino acids, incubated 2–6 h at room temperature and the appropriate dilutions of cells spread on AZ plates.

(iii) *UV irradiation*. A rapid analysis of the sensitivity of large numbers of strains to UV light was accomplished by the following procedure: (1) suspending clones of cells grown on LB agar plates in minimal broth Davis to a concentration of nearly 10^7 cells/ml, (2) spotting approximately 5 μl of cell suspension with glass rods into grid positions on to a series of LB agar plates, (3) UV irradiating the plates for exposure times that differentiate *lon sul*$^+$ from *lon sul* strains, e.g. 50–100 J/m^2.

(iv) *Chemical analyses*. Mucopolysaccharide was quantified by the methylpentose assay of Dische & Shettles (1948). Strains were spread on to minimal broth Davis plates supplemented as indicated above and grown at 37 °C for 36 h. The lawn of cells was suspended in distilled water, boiled for 10 min, and assayed. L-Rhamnose, a subunit of mucopolysaccharide, was used as the methylpentose standard. Total protein was determined by a modified Folin–Lowry assay where 0·1 ml sample of cell suspension plus 0·9 ml of 20 % Na_2CO_3–0·5 N-NaOH reagent were mixed and placed in a boiling water bath for 10 min. Upon cooling 0·1 ml of the cupric-tartrate reagent was added with 5·0 ml of water and the assay was completed by conventional Folin–Lowry procedures.

(v) *Sensitivity to antibiotics*. Strains were cultured in LB broth and harvested in exponential growth phase. Approximately 200 cells were plated on LBC solid medium with antibiotics incorporated at progressively higher concentrations. Plates were incubated at 37 °C for one day before the number of visible colonies on the plates was determined.

(vi) *Sensitivity to temperature*. Sensitivity to temperature was tested in a modified LB medium with sodium chloride concentrations of 0·0, 0·1, 0·2, 0·3, 0·4 and 0·5 %. The cell concentration for liquid medium tests was 10^7 cells/ml. When thermal sensitivity was tested on solidified LB medium approximately 200 viable cells were spread over the surface of the agar. Plates were incubated 24–48 h before the viable number was determined. Liquid cultures were incubated 12 h before microscopic examination.

3. RESULTS

(i) *The* sul *mutations of strains* Bs-8 *and* Bs-3. The *E. coli* B strains Bs-3 and Bs-8 are both UV sensitive by virtue of mutations in the *uvrB* and *uvrC* genes, respectively; consequently, the suppressive effect of *sul* mutations in these strains is not readily observable. A *fabA*-linked *sul* mutation can be demonstrated for both these strains by P1 mediated transduction into PAM 155. The transductant strain

PAM 172, that possesses the *sulA22* allele of Bs-8, has wild type resistance to UV irradiation while PAM 170, that possesses the *sulA24* allele of Bs-3, is moderately resistant to UV irradiation. This difference in UV sensitivity is also expressed in

Table 2. *Reciprocal four-factor cotransductions of* sulA *with* fabA *and* pyrD

Donor	Recipient	Selection marker	Unselected marker	Cotransduction frequency
PAM 171	PAM 155	*fabA*+	*sulA*	215/250 = 0·86
			pyrD	173/250 = 0·69
			cmlB	113/250 = 0·45
PAM 155	PAM 171	*pyrD*+	*fabA*	162/250 = 0·65
			sulA+	157/250 = 0·63
			cmlB+	182/250 = 0·73
PAM 173	PAM 155	*fabA*+	*sulA*	229/292 = 0·78
			pyrD	186/292 = 0·64
			cmlB	124/292 = 0·43
PAM 155	PAM 173	*pyrD*+	*fabA*	167/297 = 0·56
			sulA+	144/297 = 0·49
			cmlB+	206/297 = 0·69

Table 3. *Analysis of recombinant classes of a four-point, reciprocal transduction*

(I. The donor is strain PAM 173 (*cmlB pyrD fabA*+ *sulA22*) and the recipient is strain PAM 155 (*cmlB*+ *pyrD*+ *fabA sul*+). II. The donor is strain PAM 155 and the recipient is strain PAM 173.)

Transduction	Selected marker	Unselected marker				Minimal crossover events	Number of recombinants
		sulA	*fabA*	*pyrD*	*cmlB*		
I	*fabA*+	+	.	+	+	2	52/298
		+	.	+	−	4	0/298
		+	.	−	+	2	3/298
		−	.	+	+	2	58/298
		−	.	−	+	2	61/298
		−	.	+	−	4	2/298
		+	.	−	−	2	12/298
		−	.	−	−	2	110/298
II	*pyrD*+	+	+	.	+	4	0/297
		+	+	.	−	4	0/297
		+	−	.	+	2	88/297
		−	+	.	+	2	103/297
		−	−	.	+	2	15/297
		−	+	.	−	2	41/297
		+	−	.	−	2	42/297
		−	−	.	−	2	8/297

the sensitivity to MMS where PAM 172 is able to grow on LB broth agar plates containing 0·005 or 0·025 % MMS, but PAM 170 is unable to grow on plates containing 0·025 % although it grows on plates containing 0·005 % MMS.

The linkage between *fabA*, *pyrD*, *cmlB*, and both *sul* mutations was determined with the *pyrD-cmlB* derivatives of PAM 170 and PAM 172, the strains PAM 171 and PAM 173, respectively. In Table 2 it can be seen that the *sul* mutations of

strains Bs-8 and Bs-3 are highly linked to *fabA* (0·86 and 0·78) and to *pyrD* (0·63 and 0·48). Similar linkage values have been reported (Johnson & Greenberg, 1975) for the *sulA3* allele of PAM 154 except the transductional frequencies are 0·82 with the *fabA* gene and 0·31 with the *pyrD* gene. However, since George *et al.* (1975) have roughly positioned a mutation like *sul* counterclockwise of the *pyrD* gene on the chromosome we have examined in detail the recombinant classes obtained in these transductions. Table 3 presents the data from the reciprocal four-point crosses between strain PAM 173 and strain PAM 155. The minimal number of crossovers to form these recombinants has been determined assuming the gene

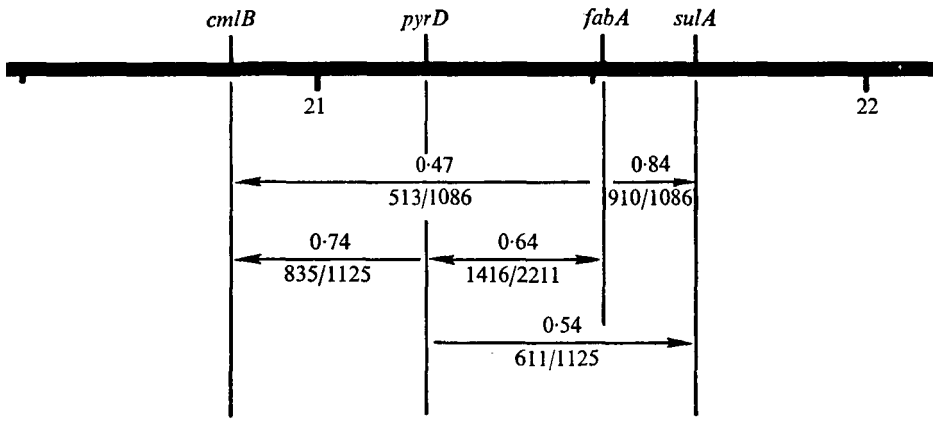

Fig. 1. Position of *sulA* and neighbouring loci on the genetic map of *Escherichia coli*. The figure is adapted from the circular map of Bachmann *et al.* (1976). The numbers above the arrow indicate the cotransduction frequencies and the fraction below the arrow the corresponding number of cotransductants over the total number of transductants examined. The arrow-heads indicate the unselected marker; double arrowheads indicate reciprocal crosses that have been averaged. Data illustrated in this figure are the summary of transductional studies using the *sulA* derivatives PAM 171, PAM 173, PAM 175 and PAM 177.

order is *cmlB-pyrD-fabA-sulA*. An analysis of the recombinational data confirms this gene order since recombinant classes hypothesized to require four crossover events occur at a very low frequency or do not occur. In the case of transduction I, one recombinant class (*fabA*$^+$ *sul*$^+$ *pyrD cmlB*) that requires a minimum of two crossover events also is observed at a very low frequency. However, the low frequency of this recombinant class is plausible due to the moderately high transductional frequency of 0·74 between the genetic markers *pyrD* and *cmlB*. Likewise, in transduction II an infrequent recombinant class formed by two crossover events is explainable by the tight linkage between *fabA* and *sulA*. An illustration of the order of these four genes with a summary of the transductional linkages for all the *sul* mutations like the *sulA23* allele of strain Bs-8 is presented in Fig. 1. These observations are in agreement with our previous mapping studies with the *sulA23* allele of PAM 154, the work of Reeve (1968), and of Foulds (1976) for other genes in this region of the chromosome. Since our laboratory and other laboratories

(Gayda *et al.* 1976; George *et al.* 1975) have observed more than one type of *sul* mutation, we have adopted the designation *sulA* for the *fabA*-linked suppressor of *lon*.

(ii) *The chromosomal location of* suf. Organessian & Organessian (1973) have reported a suppressor of the *lon* mutation located in the *trp* region of the chromosome of *E. coli* K12 that has phenotypic properties like *sul*. Table 4 presents transductional evidence that the *suf* mutation is a lesion in the *sulA* gene, being linked at a frequency of 0·55 to *pyrD* and at a frequency of 0·86 to *fabA*. The recombinational classes from this reciprocal transduction have been analysed and are essentially identical to those presented for *sulA22* in Table 3 and interpretable in the same way (analysis not presented).

Table 4. *Transductional studies with PAM 175, a* pyrD-cmlB *derivative of PAM 174, possessing the* suf (sulA29) *mutation*

Donor	Recipient	Selection marker	Unselected marker	Cotransduction frequency
PAM 175	PAM 155	*fabA*+	*sulA*	211/245 = 0·86
			pyrD	170/215 = 0·69
			cmlB	139/245 = 0·57
PAM 155	PAM 175	*pyrD*+	*cmlB*+	214/280 = 0·76
			fabA	174/280 = 0·62
			sul+	155/280 = 0·55

(iii) *Isolation and genetic characterization of suppressed K12* lon *strains*. In order to investigate the diversity of *sul* mutations, a number of suppressed *lon* derivatives of an *E. coli* K12 strain were isolated. Independently isolated clones of the *lon* strain, PAM 660, were grown in LB broth and spread on LB broth agar plates containing either 0·005 % or 0·025 % MMS. Out of a lawn of filamenting cells, colonies of non-filamenting cells arose that were selected, purified, and verified for their insensitivity to both UV irradiation and MMS. One resistant strain from each clone of PAM 660 was kept for further study. Resistant strains varied with respect to the degree of suppression of the *lon* phenotype and to their exponential growth rates. For this paper we have restricted our study to those strains which are highly resistant to both UV irradiation and MMS and which essentially grow with a generation time like that of the parental strain.

Bacteriophage P1 was grown on each of these strains and used as the DNA donor in a transduction selecting for the *fabA* region of the chromosome. Table 5 shows the strains PAM 163 and PAM 164 have a *sulA* gene highly cotransducible with the *fabA*+ gene. The other two isolates PAM 161 and PAM 162 do not possess a *fabA*-linked *sulA* gene but still possess a *proC*-linked mutation in the *lon* gene that confers UV sensitivity if transduced to strain KL398.

(iv) *Chromosomal location of* sulB25 *and* sulB26. The chromosomal location of the *sulB25* and *sulB26* alleles was approximated by Hfr matings to be slightly clockwise of the *thr-leu* region of the chromosome. This location was confirmed and precisely determined by transductional studies. Transductional data shown in

Table 5. *Transductional linkages of independently isolated methylmethane sulphonate resistant derivatives of strain PAM 660*

Donor	Recipient	Selection marker	Unselected marker	Cotransduction frequency
PAM 161	PAM 155	*fabA*+	*sulA*	0/100 = <0·01
PAM 161	KL398	*proC*+	*lon*	9/100 = 0·09
PAM 162	PAM 155	*fabA*+	*sulA*	0/200 = <0·005
PAM 162	KL398	*proC*+	*lon*	17/100 = 0·17
PAM 163	PAM 155	*fabA*+	*sulA*	93/100 = 0·93
PAM 164	PAM 155	*fabA*+	*sulA*	102/114 = 0·90
PAM 177	PAM 155	*fabA*+	*sulA*	255/299 = 0·85
PAM 177	PAM 155	*fabA*+	*pyrD*	210/299 = 0·70
PAM 177	PAM 155	*fabA*+	*cmlB*	137/299 = 0·46
PAM 155	PAM 177	*pyrD*+	*cmlB*+	233/298 = 0·78
PAM 155	PAM 177	*pyrD*+	*fabA*	174/298 = 0·58
PAM 155	PAM 177	*pyrD*+	*sul*+	155/298 = 0·52

Table 6. *Transductional linkages between* sulB25 *and neighbouring loci*

Donor	Recipient	Selection marker	Unselected marker	Cotransduction frequency
PAM 169	PAM 161	*thr*+	*leu*+	3/381 = 0·008
			sul+	0/381 = <0·003
			azi	0/381 = <0·003
			pan	0/381 = <0·003
		leu+	*thr*+	3/375 = 0·008
			sul+	177/375 = 0·47
			azi	148/375 = 0·39
			pan	0/375 = <0·003
		azi	*thr*+	0/431 = <0·002
			sul+	227/431 = 0·53
			leu+	168/431 = 0·39
PAM 168	PAM 150	*leu*+	*sulB25*	174/400 = 0·44
			pan+	2/400 = 0·005
		pan+	*sulB25*	15/384 = 0·039
			leu+	2/384 = 0·005

Table 7. *Recombinational analysis of the transduction in which PAM 169* (leu+ sul+ azi) *served as donor and PAM 161* (leu sulB25 azi+) *served as recipient.* leu+ *was the selected marker*

Recombinant classes	Minimal crossover events	Cotransduction frequency
leu+ *sulB25 azi*+	2	191/375 = 0·51
leu+ *sul*+ *azi*	2	141/375 = 0·38
leu+ *sul*+ *azi*+	2	36/375 = 0·10
leu+ *sulB25 azi*	4	7/375 = 0·02

Table 6 indicate that *sulB25* is not located between *thr* and *leu* by virtue of the fact that no *thr*$^+$ transductants are resistant to UV irradiation. The *sulB25* allele of PAM 161 was, however, readily cotransducible with the *leu* and *azi* markers with transductional frequencies of 0·45 and 0·53, respectively. *sulB25* is much more distantly linked to the *pan* gene (0·005 to 0·04) suggesting a position between the *leu* and *azi* genes on the chromosome. A recombinant analysis shown in Table 7 confirms this suggestion; in order to account for the rare recombinant *leu*$^+$ *sulB25 azi* class (7/375) the gene order must be *leu-sulB25-azi*. Nearly identical results

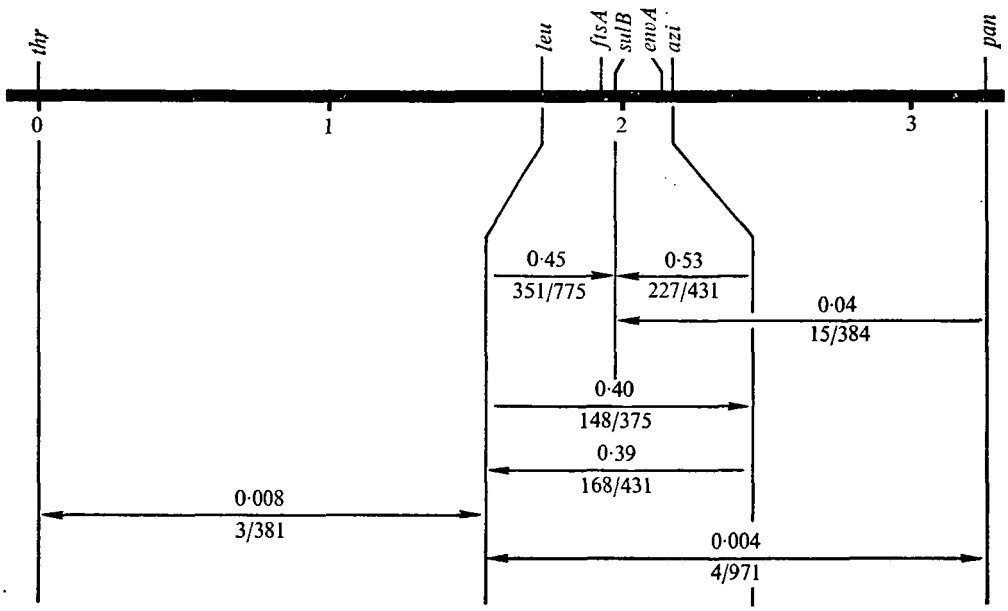

Fig. 2. Position of *sulB* and neighbouring loci on the genetic map of *Escherichia coli*. The figure is adapted from the linkage map of Bachmann *et al.* (1976). The numbers above the arrows indicate the cotransduction frequencies and those below the arrows the fraction of cotransductants in the total number of transductants. The arrowheads indicate the unselected marker.

have been obtained from mapping studies with the *sulB26* allele of PAM 162 (data not presented). We conclude, then, that both *sul* mutations are located at the same position on the chromosome that we and others (Gayda *et al.* 1976) designate *sulB*.

Our data allows *sulB* to be located on the chromosome with a fair degree of accuracy. The values for the cotransduction of *azi* with *leu*$^+$ and *leu*$^+$ with *azi* are nearly identical; consequently, the cotransductional values of *sulB* with the *azi* and *leu* genes should be comparable. We have obtained an average cotransductional frequency of 0·39 between the *leu* and *azi* genes that by the formula of Wu (1966) calculated to a chromosomal distance of 0·54 min. The recalibrated chromosomal map of Bachmann, Low & Taylor (1976) gives the value of 0·45 min for the distance between *leu* and *azi*. A summation of our linkage values for *leu-sulB* and *sulB-azi*

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gives a chromosomal map distance of 0·85 min. This value is nearly twice the value indicated by the current linkage map of the *E. coli* chromosome and significantly higher than our experimental value of 0·54. However, Wijsman has recently published transductional studies (Wijsman & Koopman, 1976) that indicate a map distance of 0·70 min between *leu* and *azi* which is nearly the mean of our results. For comparative purposes we accept the current *E. coli* map and have calibrated positions for the *sulB* gene as well as the *ftsA* and *envA* genes taking into account the disparity between our data and those of other laboratories. The chromosomal map position of *sulB* is illustrated in Fig. 2. The *sulB* gene is therefore located close to *ftsA* and between *leu* and *envA* genes on the chromosome. It should be pointed out in evaluating this data that the *azi* gene as a selecting marker was difficult to use. We obtained widely varying transductional frequencies from one transduction to the next. Only by averaging all our data have we obtained a transductional frequency between *azi* and *leu* that is in excellent agreement to the value obtained in crosses where *leu*$^+$ is the selecting marker. Transductional values using *leu*$^+$ as the selecting marker were consistently reproducible.

(v) *Analysis of independent B/r isolates.* The ease with which *sulA* and *sulB* mutations can be isolated in *E. coli* K12 raises the question as to why two classes of *sul* mutations have not been observed in this and our previous mapping study of B/r strains. To illuminate this query we isolated 20 independent B/r isolates of *E. coli* B Hill. Ten were isolated as MMS resistant and ten were isolated as crystal violet resistant derivatives of *E. coli* B. All ten isolates resistant to crystal violet and eight of the MMS resistant isolates possessed the *fabA*-linked *sulA* mutation. Two of the isolates did not possess either *sulA* or *sulB* mutations but were *lon*$^+$ revertants. Inasmuch as we have not been able to isolate the *sulB* mutation directly in *E. coli* B, we have transduced the *sulB* region of PAM 168 into the *E. coli* B strain PAM 160 with the *leu*$^+$ marker. Forty % of the *leu*$^+$ transductants were resistant to UV irradiation. Although we have not yet isolated a *sulB* derivative of *E. coli* B directly, it is apparent that the *sulB25* mutation isolated in an *E. coli* K12 genetic background is effective in suppressing the *lon* phenotype in an *E. coli* B genetic background.

(vi) *Mucopolysaccharide analysis.* One of the effects of the *lon* mutation is the derepression of several enzymes involved in sugar metabolism and the synthesis of extracellular mucopolysaccharide. All *lon* strains that we have studied in this laboratory overproduce mucopolysaccharide although the degree of UV sensitivity conferred by the *lon* mutation and the extent of mucopolysaccharide production is not strictly correlated. The extent of mucopolysaccharide synthesis is higher on minimal medium using carbohydrates as a carbon source relative to rich, complex medium or minimal medium containing non-carbohydrate carbon sources (Nakamura & Kawahara, 1974). As shown in Table 8 about a 20-fold increase in the level of mucopolysaccharide production occurs as a result of a mutation in the *lon* gene when cells are grown on a minimal medium. A secondary mutation in either the *sulA* or the *sulB* gene does not markedly repress the synthesis of mucopolysaccharide. This observation is in agreement with Gayda *et al.* (1976) who did not

observe a difference in the mucoid appearance of colonies of *lon* and *lon sul* strains grown on minimal agar plates.

(vii) *Sensitivity to antibiotics.* To determine whether *sulB* mutant strains exhibit a sensitivity to antibiotics similar to that exhibited by *envA* mutant strains the test procedures of Normark (1970) were used as described in the Methods. The parental strains PAM 660 and AB1157, the *sulB* strains PAM 161 and PAM 162, and the *sulA* strain PAM 163 were grown and plated on LBC medium with various concentrations of antibiotics to determine the minimal inhibitory concentration for colony formation. The antibiotics ampicillin, rifampicin, and actinomycin D were utilized over a range of concentrations that inhibit *envA* strains. None of the *sul*

Table 8. *Mucopolysaccharide analysis of strains grown on solid minimal medium at 37 °C for 36 h*

(Methylpentose content of the culture was determined by the method of Dische & Shettles (1948) and quantitated relative to total protein.)

Bacterial strain	Relevant genotype	Mucopolysaccharide produced $\left(\frac{\mu\text{mol rhamnose}}{\text{mg total protein}}\right)$
AB1157	*lon*$^+$ *sul*$^+$	0·13
J6271	*lon sulA29*	2·8
J6273	*lon sul*$^+$	3·7
J6275	*lon*$^+$ *sulA29*	0·22
PAM 161	*lon sulB25*	5·4
PAM 162	*lon sulB26*	4·8
PAM 163	*lon sulB27*	3·1
PAM 164	*lon sulB28*	3·0
PAM 660	*lon sul*$^+$	5·6

strains tested exhibit a significant difference in sensitivity to any of these antibiotics relative to the parental strains. The minimal inhibitory concentration for all strains was approximately 1·0 μg/ml for ampicillin, 2·5 μg/ml for rifampicin, and above 10 μg/ml for actinomycin D. Since the minimal inhibitory concentrations for *envA* strains would be much less (100-fold less for rifampicin and actinomycin D), we conclude that *sulB* is not an allele of the *envA* gene.

(viii) *Sensitivity to temperature.* Another gene located close to *sulB* on the chromsome is the *ftsA* gene. Mutations in this gene have been shown to be temperature sensitive for a step in the cell division process (Ricard & Hirota, 1973). Upon shifting the temperature for growth of a *ftsA* strain from 32 to 42 °C the cell division process stops but resumes again upon shift of the temperature back to 32 °C (Walker *et al.* 1975). The phenotypic expression of this temperature sensitivity is modified by the ionic strength of the growth medium. Under growth conditions when the sodium chloride concentrations are 0·5 % and higher the expression of the FtsA phenotype is prevented.

The temperature sensitivity of representative *sul* strains was tested in LB medium with various sodium chloride concentrations at 32 and 42 °C. The parental

sul$^+$ strains AB1157 and PAM 660 as well as the *sulA* strain PAM 163 do not exhibit any significant deviation in cellular morphology or viability at 42 °C relative to 32 °C. The two *sulB* strains PAM 161 and PAM 162 do exhibit a filamentous morphology at 42 °C under certain growth conditions. In liquid medium where the sodium chloride concentration was 0·0 to 0·1 %, more than 95 % of the cells of cultures of PAM 161 and PAM 162 grew as elongated cells at 42 °C. At progressively higher salt concentrations the proportion of elongated cells to short rods decreased until no temperature induced filamentation was observed at 0·4 % sodium chloride. On solid agar plates of LB medium with various salt concentrations that had been spread with diluted suspensions of the strains AB1157, PAM 660, PAM 161, PAM 162, and PAM 163, no significant decrease in colony forming units was observed at the lower salt concentrations at 42 °C. At salt concentrations of 0·0 to 0·1 % the strains PAM 161 and PAM 162 did, however, require 48 h instead of 24 h to give rise to countable colonies at 42 °C. Since no significant decrease in viability occurs at 42 °C for *sulB* strains it can be tentatively concluded that the *sulB* defect hinders but does not block the cell septation process at 42 °C. This observation contrasts with *ftsA* strains that cannot undergo cell division at 42 °C.

4. DISCUSSION

Two types of suppressor mutations that effect complete suppression of the UV and MMS sensitivity properties of *lon* mutations have been isolated and positioned on the *E. coli* chromosome. We agree with the suggestion of Gayda *et al.* (1976) that these suppressors be designated *sulA* and *sulB*. Recently George *et al.* (1975) have reported the chromosomal location of two suppressors of spontaneous filamentation in *tif lon* double mutants, *sfiA* and *sfiB*. One of these suppressors, *sfiB*, has an identical chromosomal location to the *sulB* gene described above. Superficially the location of the *sfiA* mutation would appear to be identical to *sulA* since both suppressors have similar linkages to the *pyrD*$^+$ gene. However, our evidence clearly indicates that *sulA* is clockwise of *pyrD* being highly cotransducible with the *fabA*$^+$ gene. George *et al.* (1975) reported recombinational data that suggests the *sfiA* gene is counterclockwise of the *pyrD* gene on the chromosome. Consequently the identity of the *sulA* and *sfiA* genes is still open to question. Further, we suppose the *sul* mutations recently described by Gayda *et al.* (1976) are identical to the *sulA* and *sulB* mutations described in this paper. However, if the *sfiA* gene should be demonstrated to be distinct from the *sulA* gene, it is possible that some of the *sulA* mutations described by Gayda *et al.* (1976) could be *sfiA*-type mutations since only transductional linkages to the *leu* marker are presently available. Finally it is clear from our studies that the *suf* gene is identical to the *sulA* gene since both genes have identical transductional linkages to *pyrD* and *fabA* and recombinational analysis establishes a position clockwise of both the *pyrD* and *fabA* genes on the chromosomal map.

We have shown that the *sulB* mutation is not likely to be an allele of the *envA* gene, since *sulB* strains are not more sensitive to antibiotics than the parental strains. James & Gillies (1973) have reported the *sul* mutation of *E. coli* B/r to

result in a greater resistance to penicillin G. We have not observed either *sulA* or *sulB* to confer greater resistance to ampicillin, actinomycin D, rifampicin, or azide. However, of considerable interest is the fact that *sulB* derivatives of *lon* strains undergo normal cell division growing in media of low ionic strength at 32 and 37 °C but undergo aberrant cell division at 42 °C. Microscopic examination reveals that cells incubated at 42 °C grow as filaments, most of which are able to undergo cell division at a reduced rate. The *sulB* and the *ftsA* mutations are located close together on the chromosome and could be lesions in the same gene; however, differences in the *ftsA* and *sulB* phenotype noted in the results section indicate that much more evidence will be required to establish this point. We do not yet know if *ftsA* mutations have any effect on the *lon* phenotype. Burdett & Murray (1974) have concluded that the *ftsA* mutation causes a lesion in an early stage of cell division involving a modification of the mucopeptide layer of the cell wall that is necessary before synthesis of the septum. It is interesting to note that located close to *ftsA* on the chromosome are a series of genes (*murC*, *E* and *F*, and *ddl*) coding for steps in the synthesis of the precursors of the mucopeptide layer of the cell wall (Wijsman, 1972). If *sulB* is an allele of *ftsA* or similar to the *ftsA* mutation, then a hydrolytic enzymatic activity could be postulated for the *sulB* gene product. The establishment of such a role for the product of the *sulB* gene would suggest a complementary role for the *lon* mutation. A defect in the *lon* mutation has been proposed to result in the accumulation of a 'repair associated division inhibitor' (George *et al.* 1975). The *sulA* and *sulB* mutations have been proposed to uncouple cell division from the control of this division inhibitor. In this model we suggest that the *sulB* gene could code for an enzymatic activity that modifies the cell wall to allow the formation of the septum. The target of the division inhibitor could be the product of the *sulB* gene. Regulatory mutations in the *sulB* gene could occur that release the activity of *sulB* gene product from the control of the cell division inhibitor. The temperature sensitivity of the two *sulB* mutations examined in this study could be ascribed to a destabilization of protein structure that occurs as a result of amino acid substitutions in a regulatory site of the protein. If *sulB* is an allele of the *ftsA* gene, then the *ftsA* phenotype could result from mutations at other sites in this gene that have a more drastic effect upon the thermal stability and function of the *sulB* gene product.

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