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Mapping of *sul*, the Suppressor of *lon* in *Escherichia coli*

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The suppressor *sul*, which is allele specific for the ultraviolet sensitivity gene *lon*, has been mapped by conjugational and transductional crosses in *Escherichia coli* K-12 and B/r. Previously, *sul* was reported to lie in the *azi* region of the *E. coli* chromosome. Evidence is presented which positions *sul* close to and clockwise of *fabA* on the *E. coli* map. Cotransductional frequencies of 31.3% were obtained between *sul* and *pyrD*, and frequencies of 82% were obtained between *sul* and *fabA*. Also, the mucoid phenotype of K-12 *lon* strains grown on minimal glucose agar plates at 37 C was not significantly effected in *sul* derivatives. No differences between the *sul* of *E. coli* B/r and that of K-12 derivatives with regard to map location or effect on mucoid production were observed.

Strain B of *Escherichia coli* is sensitive to ultraviolet radiation and becomes filamentous upon exposure to small doses of ultraviolet irradiation and numerous chemical agents that interfere with deoxyribonucleic acid synthesis (7, 9). The gene responsible for these properties, *lon*, is cotransducible with *proC* (4, 5). A similar mutation has been isolated in *E. coli* K-12 by Howard-Flanders (8) and Markovitz and Rosenbaum (10). In *E. coli* K-12, the *lon* mutation also results in a mucoid phenotype. *E. coli* B is not mucoid because of other genes present in strain B that specifically suppress mucopolysaccharide production but not ultraviolet sensitivity; one of these mucoid suppressors is linked to *his* (5). Ultraviolet-resistant derivatives of *E. coli* B, B/r, have been isolated by Witkin (15). These resistant strains do not contain reversions of the *lon* gene since an intact *lon* gene can be transduced from B/r through *proC* (3). Resistance is attributed to a gene-specific suppressor, *sul*, not linked to *lon* on the *E. coli* map (14). A previous publication from our laboratory described transductional studies indicating a 1% linkage between azide resistance and *sul* (2). Subsequent studies reported in this communication show that this interpretation of the data is wrong.

MATERIALS AND METHODS

JN broth contained 10 g of tryptone, 5 g of yeast extract, and 5 g of sodium chloride per liter. Liquid JN broth was routinely supplemented with 1 g of glucose/liter. The minimal medium was Davis minimal broth (Difco) supplemented with glucose at 5 g/liter, L-amino acids at 100 mg/liter, purines and pyrimidines at 100 mg/liter, pantothenate at 100 mg/liter, and thiamine at 10 mg/liter as needed. Plates were solidified with 1.5% agar.

Transductions were performed by the procedure of Roth (13), and the bacteriophage used, P1*b*, has been described previously (3). Conjugational experiments with strain PAM 156 as recipient and strain KL16 as donor were performed in JN broth at 37 C. Exponential cultures of donor and recipient cells were mixed at a ratio of 1:5, incubated without shaking at 37 C for 75 min, and then diluted and subjected to strong sheer forces before plating. After 75 min of mating, the *gal* marker was just beginning to enter the recipient cells at a usable frequency.

RESULTS

Attempt to demonstrate linkage between sul and markers in the azi region. The properties of *E. coli* strains used in this work are given in Table 1. When attempts were made to transduce the *sul* gene with *azi*, only one *azi* transductant out of over 500 clones examined had apparent suppressor activity. Since we had originally reported a 1% cotransduction frequency between *azi* and *sul*, other markers in the *azi* region were examined to determine the accuracy of the original studies.

Table 2 shows the cotransduction frequency between *sul* and *leu*, *pan*, and *dnaE* mutations. On the Taylor-Trotter map of the *E. coli* chromosome (14), *leu* is situated at a location 0.5 min counterclockwise of *azi*, and *pan* is located 0.8 min clockwise of *azi*. *dnaE* is located another 0.6 min clockwise of *pan* and a total of 1.4 min clockwise of *azi* (Fig. 1).

The cotransduction frequency between *leu* and *pan* was 1% in the cross between a TonA^R strain of B/r CSH (*lon sul*) and PAM 150 (*lon leu pan*). None of the *leu*$^+$ transductants (including the *leu*$^+$ *pan*$^+$ double mutants) were *sul*. Forty percent of the *pan*$^+$ transductants were TonA^R and none were *sul*. In transductions

TABLE 1. *Bacterial strains*

Strain	Sex	Genotype	Source and comments[a]
K-12 derivatives			
PAM 150	F⁻	*leu-6, proA2, pan, metA, thi-1, lacY1 galK2, ara-14, xyl-5, mlt-1, str-31, tsx-33, supE44*, Non	Complex derivative of PAM 660 (2, 3)
PAM 151	F⁻	As PAM 150 but *tonA, dnaE*	P1*b* (DY138 TonA^R) × PAM 150 → TonA^R and temperature sensitive
DY138	F⁺	*dnaE, thy, metE, endA, str*	D. Youngs; temperature-sensitive *dnaE* mutant
W620	F⁻	*thi-1, pyrD36, gltA6, galK30, str-129*	Paris strain[b]
PAM 152	Hfr	*thr-1, leu-6, thi-1, lon, tonA, tsx, supE44, lacZ4, spc*	Derivative of AB311 (E. Adelberg); *lon* is NG induced; *tonA* and *spc* are spontaneous isolates.
PAM 153	F⁻	*thi-1, pyrD36, lon, galK30, str-129*	PAM 152 × W620 → *glt*⁺ MMS^S
PAM 154	F⁻	*thi-1, pyrD36, lon, sul, cmlB, galK30, str-129*	Spontaneous MMS^R and TC^R isolates (12)
PAM 155	F⁻	*thi-1, fabA2, lon, galK30, str-129*	P1*b* (YAA1) × PAM 154 → *pyrD*⁺ and temperature sensitive
YAA1	F⁻	*thi-1, his-68, trp-45, fabA2, mlt-2, xyl-7, malA1, galK35, str-118*	J. Cronan strain[b]
KL 16	Hfr	*thi-1, rel-1*	K. B. Low strain[b]
AB1325	F⁻	*thi-1, mlt-1, xyl-5, proB, lacY purB, galK2, his-4, str-35*	E. Adelberg
B/r derivatives			
PAM 156	F⁻	As HB45 but *purB*	P1*b* (AB1325) × HB45 → penicillin counterselect for *purB*
HB45	F⁻	*thr, leu, pro, trp, his, arg, met, lac, gal, malB, lon, sul, str*, Non	H. Boyer
B/r CSH	F⁻	*lon, sul, malB*, Non	E. Witkin
WP2	F⁻	*lon, sul, trp, malB*, Non	E. Witkin
H/r 30	F⁻	*lon, sul, arg, malB*, Non	E. Witkin

[a] Abbreviations: NG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MMS, methyl methane sulfonate; TC, tetracycline. The superscripts S and R indicate sensitivity and resistance, respectively.

[b] Kindly provided by *E. coli* Genetic Stock Center, Department of Microbiology, Yale University.

between strains B/r and PAM 151 (*lon pan tonA dnaE*), 18% of the *dnaE*⁺ transductants also received the TonA^S gene of the donor but none were *sul*. These values are perhaps lower than expected, but the values in Table 2 represent cotransduction frequencies in B/r–K-12 crosses in which considerable restriction exists.

Conjugational mapping of sul. We were able to position *sul* roughly on the linkage map by matings between strains KL16 and PAM 156 (*lon sul gal purB trp*). In each mating experiment, 100 recombinants were selected for further examination. *purB* and *trp* were the most closely linked of the markers in the first cross with a linkage of 59%; this was followed with a linkage of 29% for *purB* and *sul*, whereas *gal* was clearly the most distantly linked (Table 3). The second cross in which *gal* was the selected marker showed a 31% linkage between *gal* and *sul*. This frequency is equivalent to the linkage between *purB* and *sul* in the first cross, which suggests an intermediate location on the *E. coli* map around 21 min for *sul*. The male in the second cross also had the Tsx^R phenotype, which was shown not to be inherited by any of the *gal*⁺ recombinants of this conjugation. Since Tsx^R and *lon* are 97% cotransducible (4), the above observation confirms that the *lon* region was not entering the cell and that the data presented here only represent the configuration of the *sul* gene.

An analysis of the different recombinational classes of the second mating yielded data consistent with the gene order *gal-sul-purB-trp*. The most frequent class (59/100) was a single crossover incorporating only the *gal*⁺ gene of the donor (Table 4). The next most frequent classes were recombinants that inherited the *gal*⁺ and *sul*⁺ of the donor along with or without the *purB*⁺ and *trp*⁺ of the donor; these classes totaled 31/100 recombinants. The least frequent classes were those receiving *gal*⁺ along with one

TABLE 2. *Cotransductional mapping in the azi region*

P1*b* donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency
B/r CSH TonA^R	PAM 150	*leu*$^+$	*pan*$^+$	2/200 = 1%
B/r CSH TonA^R	PAM 150	*leu*$^+$	TonA^R	0/200 = <0.5%
B/r CSH TonA^R	PAM 150	*leu*$^+$	*sul*^{a}	0/200 = <0.5%
B/r CSH TonA^R	PAM 150	*pan*$^+$	TonA^R	80/200 = 40%
B/r CSH TonA^R	PAM 150	*pan*$^+$	*leu*$^+$	2/200 = 1%
B/r CSH TonA^R	PAM 150	*pan*$^+$	*sul*	0/200 = <0.5%
B/r CSH	PAM 151	*dnaE*$^{+b}$	TonA^S	35/200 = 18%
B/r CSH	PAM 151	*dnaE*$^+$	*pan*$^+$	0/200 = <0.5%
B/r CSH	PAM 151	*dnaE*$^+$	*sul*	0/200 = <0.5%

[a] *lon sul* transductants were identified by their ability to grow on nutrient agar plates containing 0.025% methyl methane sulfonate, whereas *lon sul*$^+$ transductants were unable to multiply on this medium.

[b] *dnaE*$^+$ transductants were selected by incubating minimal broth agar plates spread with cells at 42 C.

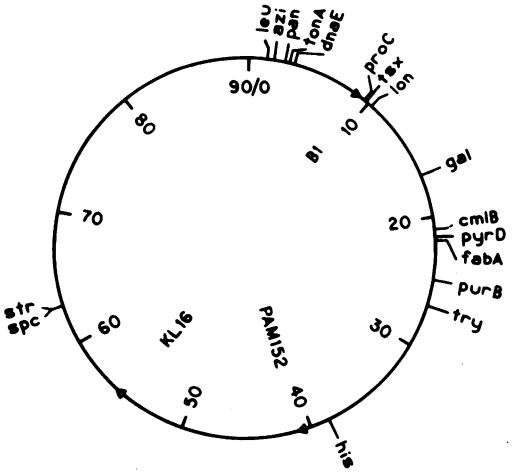

FIG. 1. *Partial map of E. coli chromosome after Taylor and Trotter (14). Arrowheads indicate point of entry of Hfr strains.*

or both the *purB*$^+$ and *trp*$^+$ genes without receiving the *sul*$^+$ gene of the donor; these classes totaled 10/100 recombinants. Since the least frequent class of recombinants is most likely explained as a double crossover, *sul* must therefore lie between *gal* and *trp*.

Transductional mapping of sul. The results of the conjugational experiments led us to test for cotransduction of *sul* with various loci in the region of *pyrD*. A *pyrD lon* strain PAM 153 was prepared from strain W620. This strain was also made *cmlB* by isolating tetracycline-resistant derivatives and *sul* by isolating methyl methane sulfonate-resistant derivatives. P1*b* was grown on strain YAA1, a temperature-sensitive *fabA* strain, which is also *cmlB*$^+$, *pyrD*$^+$, and *sul*$^+$. The *pyrD*$^+$ of strain YAA1 was transduced to strain PAM 154, and the frequency of incorporation of unselected markers is given in Table 5. The values for the linkage between *pyrD*, *cmlB*, and *fabA* were essentially in agreement with those of Cronan et al. (1). The *sul* of strain PAM 154 was found to be 31.3% cotransducibly linked to *pyrD*$^+$.

The position of *cmlB*, *pyrD*, and *fabA* relative to each other on the map has been well established by Cronan et al. (1) as shown in Fig. 2. The only question of consequence in this study is the position of *sul* relative to these genes. An analysis of the recombinational classes of the above cross indicated the position of *sul* to be clockwise of *fabA* on the circular map rather than between *pyrD* and *fabA* (Fig. 3). If one considers only the genes *pyrD*, *fabA*, and *sul*, the most frequent recombinant class, I, received only the *pyrD*$^+$ of the donor at the frequency of 58%. The next most frequent class, II, received all three genes of the donor, *pyrD*$^+$, *fabA*, and *sul*$^+$, at the frequency of 30.3%. The fact that recombinant class III, which received only the *pyrD*$^+$ and *fabA* of the donor, was only 10.7% suggests that *fabA* and *sul* are fairly closely linked. The least frequent class, IV, received the *pyrD*$^+$ and *sul*$^+$ of the donor at a frequency of 1%. Assuming that this class represents a double crossover, the gene *fabA* would of necessity be located between *pyrD* and *sul*.

Table 5 also shows data for the linkage of *sul*, *pyrD*, and *cmlB* to *fabA*. P1*b* grown on the recipient of the first cross, strain PAM 154, was used to transduce strain PAM 155 (*lon*, *fabA*) to *fabA*$^+$. The genes *fabA* and *sul* were found to be 82% linked, which confirms recombinational data of the first transductional cross that suggested tight linkage between *fabA* and *sul*.

Map position of the sul gene of B/r strains. The above mapping studies were performed with *E. coli* K-12 strains with two spontane-

TABLE 3. *Conjugational mapping in the gal-purB region*

Mating	Selected markers	Frequency (%) unselected markers				
		trp$^+$	*purB*$^+$	*sul*$^+$	*gal*$^+$	Tsx^R
KL16 × PAM 156	*purB*$^+$ *str*	59	100	29	13	
KL16 Tsx^R × PAM 156	*gal*$^+$ *str*	20	24	31	100	0

TABLE 4. *Recombinational classes obtained from mating KL16 with PAM 156*

Genotype of recombinants		No. of recombinants/100 examined
Selected markers	Unselected markers	
gal$^+$ *str*	*sul purB trp*	59
gal$^+$ *str*	*sul*$^+$ *purB trp*	14
gal$^+$ *str*	*sul*$^+$ *purB*$^+$ *trp*$^+$	12
gal$^+$ *str*	*sul*$^+$ *purB*$^+$ *trp*	5
gal$^+$ *str*	*sul purB*$^+$ *trp*$^+$	5
gal$^+$ *str*	*sul purB trp*$^+$	3
gal$^+$ *str*	*sul purB*$^+$ *trp*	2

TABLE 5. *Cotransductional mapping of the pyrD-fabA region*

P1*b* donor	Recipient	Selected marker	Unselected marker	Cotransduction frequency
YAA1[a]	PAM 154	*pyrD*$^+$	*cmlB*$^+$[b]	174/300 = 58.0%
YAA1	PAM 154	*pyrD*$^+$	*fabA*	123/300 = 41.0%
YAA1	PAM 154	*pyrD*$^+$	*sul*$^+$	94/300 = 31.3%
PAM 154	PAM 155	*fabA*$^+$[c]	*sul*	164/200 = 82.0%
PAM 154	PAM 155	*fabA*$^+$	*pyrD*	107/200 = 53.5%
PAM 154	PAM 155	*fabA*$^+$	*cmlB*	55/200 = 27.5%

[a] Because of the temperature-sensitive *fabA* marker of YAA1, all steps in the transductional cross P1*b* (YAA) × PAM 154 were performed at 30 C.

[b] The CmlB phenotype was scored on minimal broth agar plates containing 1 μg of tetracycline per ml.

[c] FabA$^+$ transductants were selected in the cross P1*b* (PAM) 154 × PAM 155 at 42 C on minimal broth agar plates.

ously isolated *sul* derivatives. We then examined representative B/r strains to confirm the identity of the genes involved. Two B/r strains used in our previous studies (2, 3), B/r CSH and PAM 156, as well as strains WP2 and H/r30 were examined for a *fabA*-linked *sul*. All four strains had *sul* genes 80 to 90% cotransducible with *fabA*. We must conclude, therefore, that previous studies which mapped *sul* in the *azi* region were erroneous and that the *sul* of B/r strains commonly in use lies very close to *fabA*.

Mucopolysaccharide production in sul strains. The mucopolysaccharide production of *lon* derivatives of *E. coli* K-12 was appraised by the appearance of colonies growing on minimal broth agar plates at 37 C. Under these conditions, the *lon* strains PAM 150, PAM 152, and PAM 155 as well as the *lon sul* strain PAM 154 were equally mucoid in appearance and readily discernible from *lon*$^+$ *sul*$^+$ strains, which were essentially nonmucoid in appearance. Furthermore, all *lon sul* derivatives of PAM 155 obtained by transducing the *sul* of B/r strains with *fabA*$^+$ formed mucoid clones. We must conclude, therefore, that *sul* has no significant suppressive effect on mucopolysaccharide synthesis.

DISCUSSION

The *sul* gene is 82% cotransducibly linked to *fabA* and is located clockwise of *fabA* on the Taylor-Trotter chromosomal map of *E. coli* (Fig. 2). Foulds (6) has mapped a *fabA*-linked mutation, *tolG*. *tolG* is 76% cotransducibly linked to *fabA*, being placed counterclockwise of *fabA* on the *E. coli* map. These strains are sensitive to bacteriocin JF246 and are more sensitive than *tolG*$^+$ strains to ethylenediaminetetraacetic acid and eosin Y. Although we have mapped *sul* clockwise of *fabA*, the sensitivity of *lon sul*, *lon sul*$^+$, and *lon*$^+$ *sul*$^+$ strains of *E. coli* K-12 to ethylenediaminetetraacetic acid and eosin Y has been determined according to the procedure of Nagel de Zwaig and Luria (11). No differences in the sensitivity of these three strains could be demonstrated (unpublished observations). Consequently, *tolG* and *sul* are not identical nor do they have similar properties.

Also, we are presently examining a series of independently isolated methyl methane sulfonate-resistant *lon* strains for *fabA*-linked *sul* genes. All isolates thus far examined have *sul* genes linked to *fabA;* no *sul* gene has been found linked to genetic loci in the *azi* region of the chromosome. M. G. Ogannessian and H. G. Ogannessian (Proc. 13th Int. Cong. Genet., abstr. p s200, 1973) have reported the characterization of a suppressor for *lon*, which is referred to as *suf*. Like *sul*, the *suf* mutant suppresses sensitivity to ultraviolet irradiation and

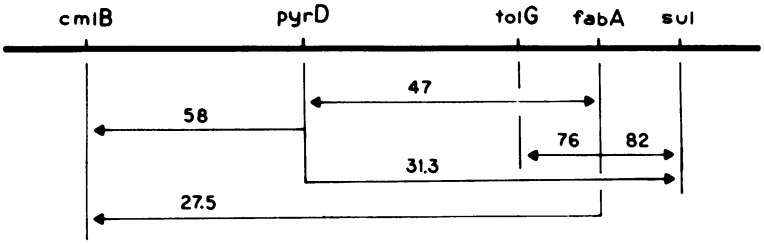

FIG. 2. *Position of sul on the genetic map of E. coli. The figure is adapted from the circular map of Taylor and Trotter (14). The numbers represent cotransduction percentages. The arrowheads indicate the unselected marker; double arrowheads indicate that reciprocal crosses have been done and that the cotransduction percentages have been averaged. The data for fabA-tolG are from the work of Foulds (6).*

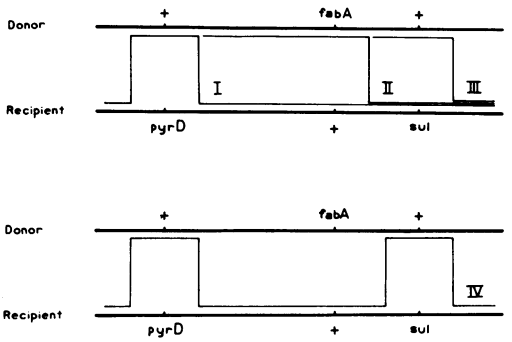

FIG. 3. *Recombinational classes of the P1b-mediated transduction where YAA1 was the donor and PAM 154 was the recipient.*

filamentation in *lon* strains without affecting mucopolysaccharide overproduction. This mutation has been shown by Ogannessian and Ogannessian to lie in the *trp* region of the chromosome and it is, in all probability, identical to *sul*.

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LITERATURE CITED

1. Cronan, J. E., Jr., D. F. Silbert, and D. L. Wulff. 1972. Mapping of the *fabA* locus for unsaturated fatty acid biosynthesis in *Escherichia coli*. J. Bacteriol. **112**:206-211.
2. Donch, J., Y. W. Chung, M. H. L. Green, J. Greenberg, and G. Warren. 1971. Genetic analysis of *sul* mutants of *Escherichia coli* B. Genet. Res. **17**:185-193.
3. Donch, J., Y. S. Chung, and J. Greenberg. 1969. Locus for radiation resistance in *Escherichia coli* strain B/r. Genetics **61**:363-370.
4. Donch, J., and J. Greenberg. 1968. Genetic analysis of *lon* mutants of strain K-12 of *Escherichia coli*. Mol. Gen. Genet. **103**:105-115.
5. Donch, J., and J. Greenberg. 1970. Protection against lethal effect of ultraviolet light on *Escherichia coli* by capsular polysaccharide. Mutat. Res. **10**:153-155.
6. Foulds, J. 1974. Chromosomal location of the *tolG* locus for tolerance to bacteriocin JF246 in *Escherichia coli* K-12. J. Bacteriol. **117**:1354-1355.
7. Hill, R. F., and R. R. Feiner. 1964. Further studies of ultraviolet-sensitive mutants of *Escherichia coli* strain B. J. Gen. Microbiol. **35**:105-114.
8. Howard-Flanders, P., E. Simson, and L. Theriot. 1964. A locus that controls filament formation and sensitivity to radiation in *Escherichia coli* K-12. Genetics **49**:237-246.
9. James, R., and N. E. Gillies. 1973. The sensitivity of suppressed and unsuppressed *lon* strains of *Escherichia coli* to chemical agents which induce filamentation. J. Gen. Microbiol. **76**:429-436.
10. Markovitz, A., and N. Rosenbaum. 1965. A regulator gene that is dominant on an episome and recessive on a chromosome. Proc. Natl. Acad. Sci. U.S.A. **54**:1084-1091.
11. Nagel de Zwaig, R., and S. E. Luria. 1967. Genetics and physiology of colicin-tolerant mutants of *Escherichia coli*. J. Bacteriol. **94**:1112-1123.
12. Reeve, E. C. R. 1968. Genetic analysis of some mutations causing resistance to tetracycline in *Escherichia coli* K-12. Genet. Res. **11**:303-309.
13. Roth, J. R. 1970. Genetic techniques in studies of bacterial metabolism, p. 3-35. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 27A. Academic Press Inc., New York.
14. Taylor, A. L., and C. D. Trotter. 1972. Linkage map of *Escherichia coli* strain K-12. Bacteriol. Rev. **36**:504-524.
15. Witkin, E. M. 1947. Genetics of resistance to radiation in *Escherichia coli*. Genetics **32**:221-248.