JOURNAL OF BACTERIOLOGY, July 1970, p. 32-36
Vol. 103, No. 1

Genetic Mapping and Dominance of the Amber Suppressor, *Su1* (*supD*), in *Escherichia coli* K-12[1]

EDWIN P. HOFFMAN AND ROBERT C. WILHELM

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

Received for publication 10 April 1970

In this report *Su1* (*supD*), which is known to suppress "amber" mutations by means of a specific transfer ribonucleic acid, has been mapped relative to *his* and *uvrC* and has been found to be located at about 37.5 min on the Taylor and Trotter genetic map of the *Escherichia coli* K-12 chromosome. In addition, $Su1^+$ has been shown to be dominant over $Su1^-$, which supports the idea that *Su1* is the structural gene for the suppressing transfer ribonucleic acid.

Extensive biochemical studies in this and other laboratories (1, 5, 8), with in vitro protein-synthesizing systems, have shown that *Su1* (*supD*) suppression of "amber" mutations was effected through a specific suppressing transfer ribonucleic acid (RNA). These results suggested that $Su1^+$ might be the structural gene for the suppressing transfer RNA. If it is, $Su1^+$ must produce a diffusible product, and $Su1^+$ should be genetically dominant over $Su1^-$. Signer et al. (11) have shown that $Su2^+$ (*supD*) is at least partially dominant over $Su2^-$. The present studies establish the dominance of $Su1^+$ over $Su1^-$.

Two laboratories (6, 11) have shown that *Su1* (*supD*) maps near *his* by recombination linkage (80%) and by the co-transduction frequency of the two genes by P1 phage [8%; Signer et al. (11); 6%, Garen et al. (6)]. In this report we map the position of *Su1* relative to *his* and *uvrC*.

MATERIALS AND METHODS

Bacterial strains. Tables 1 and 2 list the source and genotype of the various bacterial strains used in this report. The position of markers is shown in Fig. 1.

Nomenclature. The genetic system of nomenclature used in this paper is that recommended by Demerec et al. (3) with the following exceptions. (i) The symbols *Su1* and *Su2* are used for two suppressors of the N1 or "amber" nonsense codon, since these symbols have been previously published (1, 5, 6). To conform with the system of Demerec et al., the symbols *supD* and *supB* have been proposed for these loci. (ii) Since the mutant allele of *Su1* actively suppresses "amber" mutations, it is referred to as the $Su1^+$ allele; the non-suppressing, wild-type allele is referred to as $Su1^-$. In all other instances, the symbol "+" indicates the wild-type allele. (iii) Str^s refers to the wild-type allele of *str*, and mutations to str^r confer resistance to streptomycin. (iv) The symbol val^s refers to the wild-type allele of *val*, and mutations to val^r allow the strain to grow when valine, but not isoleucine, is present in the growth medium.

Procedures. Conjugation experiments were conducted as follows. The Hfr strain was grown in LB broth (see reference 6) without shaking, to a cell density of 10^8 cells/ml at 37 C, and the F^- strain was grown in LB broth with shaking to a cell density of 3×10^8 cells/ml at 37 C. The two strains were mixed in equal volumes, and the suspension was incubated without shaking for an appropriate time period (usually 2 to 3 hr). The culture was then centrifuged, the precipitate was suspended in saline (0.85% NaCl), and appropriate dilutions were plated on selective media (TG + various additions, see reference 6).

P1 transductions. P1 transductions were accomplished by using P1v phage obtained from A. Garen. The donor strain was infected when it reached a cell density of 10^8 cells/ml in LB broth containing 0.01 M $CaCl_2$ (in a 37 C incubator shaker) at a multiplicity of infection (MOI) of 0.02. After noticeable lysis, the supernatant fluid was sterilized by passing through a membrane filter (Millipore Corp., Bedford, Mass.), and the phage were titered (about 10^{10} phage/ml). The recipient culture was grown overnight in LB broth containing 0.01 M $CaCl_2$ at 37 C with shaking, and was infected at a MOI of 0.2 for 20 min. The solution was centrifuged, the precipitate was washed with saline, and appropriate dilutions were plated on selective media.

Suppressor assay. The suppressing character of the various strains was assayed as follows. The enzyme alkaline phosphatase is induced when alkaline phosphatase-positive strains are grown in a medium which is low in orthophosphate (7). The presence of this enzyme in growing colonies can be detected by using the α-napthyl acid phosphate assay discussed below. Strains which contain a nonsense mutation in the structural gene (*phoA*) do not produce the enzyme unless a suppressor gene is present. The S26 allele of

[1] This report is part of a thesis presented by E. P. H. to Yale University in partial fulfillment of the requirements for a Ph.D. degree.

TABLE 1. *List of bacterial strains*

Strain	Sex[a]	Genotype												Source[b]
		Sul	*met*	*his*	*trp*	*arg*	*thi*	*thy*	*phoA*	*uvrC*	*recA*	*str*	*val*	
KL96	Hfr(*40*)	−	+	+	+	+	−	+	+	+	+	s	s	B. Low
KL96-A	Hfr(*40*)	−	+	−	+	+	−	+	+	+	+	s	s	Penicillin selection on KL96
KL96-B	Hfr(*40*)	+	+	+	+	+	−	+	+	+	+	s	s	P1 co-transduction of *his*$^+$, *Sul*$^+$ from S26R1e into KL96-A
KL16-99	Hfr(*57*)	?	+	+	+	+	+	+	+	+	1	s	s	B. Low
AB2435	Hfr(*57*)	−	+	−	+	−	+	+	+	−	+	s	s	P. Howard-Flanders
S26R1e	Hfr(*15*)	+	+	+	+	+	+	+	S26	+	+	s	s	A. Garen
HW-1A1	F$^-$	−	+	−	−	+	+	−	S26	+	+	r	r	A. Garen; made valine-resistant and thymine-requiring
HW-1A2	F$^-$	−	+	−	−	+	+	+	S26	+	1	r	r	Thy$^+$, Str^r recombinant from KL16-99 × HW-1A1
HW-1A3	F$^-$	−	+	−	+	+	+	+	S26	−	+	r	r	Trp$^+$Str^r recombinant from AB2435 × HW-1A
HW-1C1	F$^-$	+	+	−	+	+	+	+	S26	+	+	r	r	Trp$^+$Str^r recombinant from S26R1e × HW-1A
HW-1C2	F$^-$	+	+	−	+	+	+	−	S26	+	+	r	r	HW-1C1 requiring thymine
HW-1C3	F$^-$	+	+	−	+	+	+	+	S26	+	1	r	r	Thy$^+$Str^r recombinant from KL16-99 × HW-1C2
HW-5A1	F$^-$	−	−	−	+	+	+	−	S26	+	+	r	r	A. Garen; made valine-resistant and thymine-requiring
HW-5A2	F$^-$	−	−	−	+	+	+	+	S26	+	1	r	r	Thy$^+$Str$^+$ recombinant from KL16-99 × HW-5A1

[a] Numbers in parentheses, (*40*), (*57*), (*15*), refer to points of origin on the Taylor-Trotter (13) map of the *Escherichia coli* chromosome for the transfer of the Hfr chromosome. Also see Fig. 1.

[b] Penicillin selection was accomplished by following the procedure described by Curtiss et al. (2). The strains requiring thymine were isolated by the procedure described by Stacey and Simson (12).

TABLE 2. *Episomal strains*

Strain	Sex[a]	Genotype								Source[b]
		Episomal		Chromosomal						
		his	*Sul*	*his*	*trp*	*Sul*	*phoA*	*recA*	*str*	
WH-1	F′	+	+	−	−	−	S26	1	r	*his*$^+$*Sul*$^+$ recombinant from KL96-B and HW-1A2
WH-1A	F′	−	+	−	−	−	S26	1	r	Penicillin selection on WH-1
WH-1B	F′	+	−	−	−	−	S26	1	r	Co-transduction of *his*$^+$*Sul*$^-$ markers into WH-1A

[a] See footnote *a*, Table 1.

[b] See footnote *b*, Table 1.

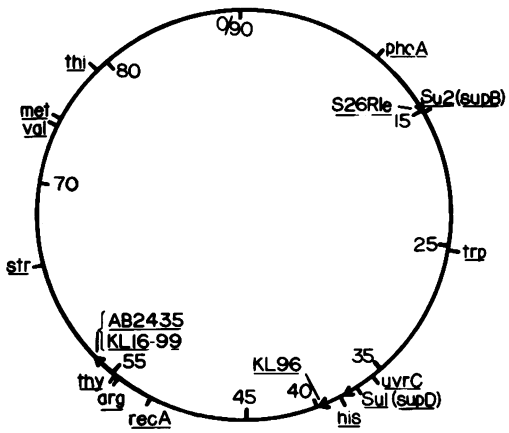

FIG. 1. *Linkage map of Escherichia coli K-12. See Table 1 for explanation of nomenclature used. Arrows indicate the direction of chromosomal transfer.*

phoA was determined to be an N1 nonsense codon since it was suppressible by *Sul*$^+$ (6). Therefore, the presence of active alkaline phosphatase in a strain carrying *phoAS26* indicates the presence of the active *Sul*$^+$ gene.

Alkaline phosphatase assay. The presence of alkaline phosphatase in growing colonies was detected by the procedure of A. Garen (*personal communication*). Colonies which were grown on plates low in orthophosphate were sprayed with a solution containing 2 mg of α-naphthyl acid phosphate per ml in 1 M tris(hydroxymethyl)aminomethane-hydrochloride, *p*H 8.0, and immediately thereafter with a solution containing 10 mg/ml of fast red violet salts (UN 1325, Verona Dyestuffs, Union, N.J.) to which 0.025 ml of 6% borate was added per ml. A brown ring forms around colonies that produce enzymatically active alkaline phosphatase. Colonies with no active alkaline phosphatase do not react and appear white.

Preparation of episomes. Episome-donating strains were prepared by the technique of Low (10). An Hfr strain which transferred early the gene to be placed on the episome was mated for short times with an F$^-$ strain containing a *recA*$^-$ gene. A large fraction of the recombinants were partial diploids, maintaining stable episomes with the desired gene.

RESULTS

Dominance of Sul$^+$ over Sul$^-$ in F′ partial diploids. A strain containing a *his*$^+$,*Sul*$^+$ episome was produced as follows. The Hfr strain, KL96-B (*his*$^+$,*Sul*$^+$,*str*^{s}) and HW-1A2 (*his*$^-$,*trp*$^-$,*Sul*$^-$, *phoAS26*,*recA1*,*str*^{r}) were mated for 30 min, and His$^+$,Str^r partial diploids were selected. The strains containing *his*$^+$ on the episome were tested independently to determine whether *Sul*$^+$ was also present on the episome by using P1 transduction. P1 phage were grown on the episome-donating strains, and then the recipient strain HW-1A (*his*$^-$,*Sul*$^-$) was infected. Transductant colonies were required to be His$^+$. The presence of *Sul*$^+$ was determined by using the alkaline phosphatase spray assay. Forty-five *his*$^+$ episomal strains were tested before one was found which contained *Sul*$^+$ as well. The episome which had been shown to contain *his*$^+$ and *Sul*$^+$ was transferred from this strain, WH-1 (*his*$^-$, *trp*$^-$,*Sul*$^-$,*phoAS26*,*recA1*/*F*,*his*$^+$,*Sul*$^+$), into strain HW-5A2 (*met*$^-$,*his*$^-$,*Sul*$^-$,*phoAS26*,*recA1*) by selecting His$^+$,Trp$^+$ recombinants. One-hundred per cent of these colonies (greater than 10,000) were brown-ringed (*Sul*$^+$) as determined by the alkaline phosphatase spray assay. Twenty recombinants were tested to insure that the episome was present and could be transferred during conjugation. These results indicate clearly that *Sul*$^+$ is dominant over *Sul*$^-$.

Enzyme assays, made by Hoffman and Wilhelm (8*a*), indicated that the alkaline phosphatase activity of strains WH-1 (*phoAS26*,*Sul*$^-$/*F*,*Sul*$^+$) and HW-1C3 (*phoAS26*,*Sul*$^+$) were identical. Strain HW-1A2 (*phoAS26*,*Sul*$^-$) had no detectable alkaline phosphatase activity.

However, it is possible that this effect, instead of being due to the presence of *Sul*$^+$, could be due to other genes present on the episome which function in the presence of *Sul*$^+$ or *Sul*$^-$. Therefore, an episome containing *his*$^+$,*Sul*$^-$ carried by strain WH-1B (identical with strain WH-1 except for modified episome) was prepared and transferred into strains HW-1C3 (*his*$^-$,*Sul*$^+$) and HW-1A2 (*his*$^-$,*Sul*$^-$). His$^+$ colonies were selected to insure the presence of the episome in each case. The His$^+$ colonies (greater than 10,000) were tested for their suppressor character. All colonies resulting from the mating of HW-1C3 were brown-ringed (Su$^+$), whereas those from the mating of HW-1A2 were white (Su$^-$).

These experiments confirm that *Sul*$^+$ is dominant over *Sul*$^-$.

Location of Sul relative to his. To determine the location of *Sul* relative to *his*, we produced many episome-donating strains in crosses between KL96-B and HW-1A2 by the technique of Low (10). The order of genes can be determined by noting the frequency with which the episomes, which contained one of the two genes, carried the second gene as well. If the gene order of strain KL96-B is F,*his*,*Sul*, episomes which contain *his* need not have *Sul*, but those containing *Sul* must have *his* as well. Results (Table 3) show that *Sul* must be further from the F integration site than *his*.

Gene order of his,Sul,uvrC. Howard-Flanders et al. (9) have shown that *uvrC* is located between *his* and *trp* and lies within several minutes of *his*.

Since the above evidence indicates that *Sul* is also between *his* and *trp* and close to *his*, the position of *Sul* can be determined more accurately if this gene is located relative to *uvrC*. P1 transduction studies to establish the gene order of *his*,*Sul* and *uvrC* were carried out. If *his*$^+$ is transduced from a *his*$^+$,*Sul*$^+$,*uvrC*$^+$ donor strain into a *his*$^-$, *Sul*$^-$,*uvrC*$^-$ strain, and a second donor gene is selected for as well, the number of transductant colonies maintaining the third donor gene can be determined. If this third gene is located between the first two genes, almost all transductant colonies selected to have the first two genes of the donor will also have the third gene of the donor. If the third gene falls outside the first two, many of the transductants will not contain the third gene (Table 4). These results show the gene order to be *his*,*Sul*,*uvrC*.

Linkage frequencies determined in appropriate conjugation experiments support the transduction data indicating the gene order to be *his*,*Sul*,*uvrC*. Strain S26R1e (*his*$^+$,*Sul*$^+$,*phoAS26*,*uvrC*$^+$, *str*^{s},λ^-) was mated with HW-1A3 (*his*$^-$,*Sul*$^-$, *phoAS26*,*uvrC*$^-$,*str*^{r},λ^-), and His$^+$Str^r recombinants that were either *Sul*$^+$ or *Sul*$^-$ were selected. The linkage of *his*$^+$ and *Sul*$^+$ was determined to be 80%, confirming earlier results (6). The *Sul*$^+$ or *Sul*$^-$ recombinants were then tested to determine the frequency of the presence of *uvrC*$^-$. The results shown in Table 5 indicate a linkage of *uvrC* with *Sul* of about 77%.

DISCUSSION

The gene order *his*,*Sul*,*uvrC* has been established. The coinheritance frequencies between *his* and *Sul* (80%) and between *uvrC* and *Sul* (77%) suggest that *Sul* might be located about midway between these two genes and therefore at about 37.5 min on the Taylor-Trotter (13) map. The position of *Sul* (*supD*) appears to be quite close to the location of a missense suppressor, *supH* (4), proposed by Howard-Flanders et al. (9) in time-of-entry experiments with *uvrC* as a comparative marker. Experiments are in progress to determine whether the two genes are identical.

The dominance of *Sul*$^+$ over *Sul*$^-$ has been demonstrated by the use of episomal strains. This evidence indicates that *Sul*$^+$ makes a diffusible gene product, which further supports the suggestion that *Sul* is a structural gene for suppressing transfer RNA. It is still possible that *Sul* could make another type of diffusible product (modifying enzyme, etc.) and a second gene could produce suppressor transfer RNA; however, no published studies have established the existence of such a second gene.

TABLE 3. *Analysis of episome-donating strains produced by mating KL96-B with HW-1A2*[a]

Gene selected	No. of episomal strains isolated	Second gene tested	No. with second gene
Sul$^+$	22	*his*$^+$	22
his$^+$	24	*Sul*$^+$	0

[a] Hfr—*F*,*his*$^+$,*Sul*$^+$,*str*^{s} × F$^-$—*his*$^-$,*Sul*$^-$, *recA*$^-$,*str*^{r},*phoAS26*. Episome-donating strains were prepared by mating KL96-B and HW-1A2 in broth for 10 min. The mating was then interrupted by using a Vortex mixer, and recombinants that were Str^r and contained either *Sul*$^+$ or *his*$^+$ as indicated were selected. The presence of the second marker was then tested. The results were reproduced by using episome-donating strains isolated from a separate cross of the two parental strains, with an independent isolate of the Hfr.

TABLE 4. *Co-transductants containing his*$^+$ *and a second donor gene; tested for a third donor gene*[a]

Gene required	Gene tested	Total colonies	No. with gene tested
Sul$^+$	*uvrC*$^+$	36	0
uvrC$^+$	*Sul*$^+$	7	7
Only *his*$^+$		545	

[a] P1v was grown on S26R1e (*his*$^+$,*Sul*$^+$,*uvrC*$^+$) and strain HW-1A3 (*his*$^-$,*Sul*$^-$,*uvrC*$^-$) was subsequently infected. Colonies containing His$^+$ transductants and a second gene as indicated were selected. The presence of the third gene was determined by testing the colonies for sensitivity to ultraviolet light (*uvrC*) or for the presence of alkaline phosphatase (*Sul*). Transductant colonies containing *uvrC*$^+$ were selected by using a modification of the technique of Howard-Flanders et al. (8). The plates containing the transductant colonies were subjected to short pulses (80 ergs/mm^2) of ultraviolet light every 40 min after plating for 120 min, and thereafter at 3, 6, and 18 hr after mating. The surviving colonies were then individually tested to insure that they were *uvrC*$^+$. All strains used were free of prophage.

TABLE 5. *Recombination frequency between Sul and uvrC*[a]

Gene selected	Colonies	No. *uvrC*$^-$	Per cent (*uvrC*$^-$/total colonies)
Sul$^+$	200	45	22
Sul$^-$	110	85	77

[a] Strains S26R1e and HW-1A3 were mated as described in the text. His$^+$,Str^r colonies were randomly selected as either *Sul*$^+$ or *Sul*$^-$ as indicated. These colonies were then tested to determine ultraviolet sensitivity (*see* Table 4).

ACKNOWLEDGMENTS

We thank Gertrud von Hesberg and Daralyn Hodgetts for excellent technical aid.

This investigation was supported by grants GB-3100 and GB-7147 from the National Science Foundation. One of us (E. P. H.) was a predoctoral trainee (Public Health Service grant GM-00711).

LITERATURE CITED

1. Capecchi, M. R., and G. N. Gussin. 1965. Suppression *in vitro*: identification of a serine-sRNA as a "nonsense" suppressor. Science **149**:417–422.
2. Curtiss, R. III, L. J. Charamella, C. M. Berg, and P. E. Harris. 1965. Kinetic and genetic analyses of D-cycloserine inhibition and resistance in *Escherichia coli*. J. Bacteriol. **90**: 1238–1250.
3. Demerec, M., E. A. Adelberg, A. J. Clark, and P. E. Hartman. 1966. A proposal for a uniform nomenclature in bacterial genetics. Genetics **54**:61–76.
4. Eggertsson, G., and E. Adelberg. 1965. Map positions and specificities of suppressor mutations in *Escherichia coli* K-12. Genetics **52**:319–340.
5. Engelhardt, D., R. Webster, R. C. Wilhelm, and N. Zinder. 1965. *In vitro* studies on the mechanism of suppression of a nonsense mutation. Proc. Nat. Acad. Sci. U.S.A. **54**:1791–1797.
6. Garen, A., S. Garen, and R. C. Wilhelm. 1965. Suppressor genes for nonsense mutations I. The *Su-1*, *Su-2* and *Su-3* genes of *Escherichia coli*. J. Mol. Biol. **14**:167–178.
7. Garen, A., and C. Levinthal. 1960. A fine-structure genetic and chemical study of the enzyme alkaline phosphatase of *Escherichia coli*. I. Purification and characterization of alkaline phosphatase. Biochim. Biophys. Acta **38**:470–483.
8. Gesteland, R. F., W. Salser, and A. Bolle. 1967. *In vitro* synthesis of T4 lysozyme by suppression of amber mutations. Proc. Nat. Acad. Sci. U.S.A. **58**:2036–2042.

8*a*. Hoffman, E. P., and R. C. Wilhelm. 1970. Effect of *Sul*$^+$ gene dosage on the production of suppressed alkaline phosphatase in *Escherichia coli* K12. J. Mol. Biol. **49**:241–244.

9. Howard-Flanders, P., R. Boyce, and L. Theriot. 1966. Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53**:1119–1136.
10. Low, B. 1968. Formation of merodiploids in matings with a class of *REC*$^-$ recipient strains of *Escherichia coli* K12. Proc. Nat. Acad. Sci. U.S.A. **60**:160–167.
11. Signer, E. R., J. R. Beckwith, and S. Brenner. 1965. Mapping of suppressor loci in *Escherichia coli*. J. Mol. Biol. **14**:153–166.
12. Stacey, K. A., and E. Simson. 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554–555.
13. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. **31**:332–353.