JOURNAL OF BACTERIOLOGY, Mar. 1972, p. 1125-1133

Vol. 109, No. 3
Printed in U.S.A.

Close Linkage Between *Ochre* and Missense Suppressors in *Escherichia coli*

E. ORIAS, T. K. GARTNER, J. E. LANNAN, AND M. BETLACH

Department of Biological Sciences, University of California at Santa Barbara, Santa Barbara, California 93106

Received for publication 18 August 1971

It was previously shown that the *ochre* suppressor mutation *sup15B* in *Escherichia coli* determines the accumulation of altered 30*S* ribosomal subunits and the presence of altered transfer ribonucleic acid (tRNA) capable of suppressing in vitro the UAG codon. This mutation has been mapped in the present study by means of conjugation and transduction experiments. After establishing the location of *sup15B* near *argH*, the following order was determined for the markers tested: *metB-argH-(sup15B, supA36)-rif-thi*. A comparison of location, growth rate, and suppressor pattern determined by *sup15B* and *supM* indicates the high probability that both suppressor mutations are identical. This study has also yielded a more precise location for the rifampin resistance gene. The most interesting finding is the very close (if not adjacent) location of the suppressor mutations *sup15B* and *supA36*, both of which determine tRNA alterations.

Strain 15B of *Escherichia coli* was isolated in our laboratory from strain 2320 (λ) (16) and differs from strain 2320 (λ) by a single mutation (*sup15B*) which determines (i) in vivo *ochre* suppressor activity (16), (ii) the accumulation of altered 30*S* ribosomal subunits (28), and (iii) the presence of altered transfer ribonucleic acid (tRNA) which can suppress in vitro (18) the UAG codon present in mutant *sus13* of bacteriophage f2 (33).

In further genetic studies of *sup15B*, we have performed transductions which have revealed the order of three markers in the *argH-thi* interval of the *Escherichia coli* genetic map (31). The markers studied were *metB*, *argH*, *sup15B*, *supA36* (5, 7, 20), *supM* (13, 14), *rif* (2, 3, 15, 23, 32), and *thi*.

The evidence presented below indicates that the order of the markers is *metB-argH-(sup15B, supA36)-rif-thi*, and that *supM* and *sup15B* probably are identical mutations of independent origin. The most interesting finding is the very close linkage between *sup15B* and *supA36;* these two suppressor mutations, each known to determine the presence of altered tRNA, are at least 99% cotransducible by phage P1Kc.

MATERIALS AND METHODS

Bacterial strains. The source and genotype of each bacterial strain used are listed in Table 1. Recombinant strains were constructed by conjugation or transduction.

A number of bacterial mutants were isolated and used in this study. Their characteristics and methods of isolation are described below.

Isolation of fast-growing derivatives of strain 15B. Strain E31 was isolated as a fast-growing derivative of strain 15B to circumvent the problems caused by the slow growth of strain 15B (16) and its frequent replacement by a variety of faster growing mutants. The mutation which gave rise to strain E31 is very closely linked to the 15B *ochre* suppressor mutation (more than 95% cotransducible with it) and results in a nearly normal growth rate for strain E31. Strains E54 and S30 are, respectively, an EMS-induced and a spontaneous mutant of strain 15B, also isolated on the basis of their faster growth rate on tryptone plates at 37 C. The mutation which gave rise to strain E54 is also very closely linked to the *sup15B* mutation (more than 95% cotransduction), whereas the new mutation in strain S30 is unlinked (less than 5% cotransducible) with the *sup15B* mutation. Strains E31, E54, and S30 still have readily detectable *ochre* suppressor activity.

Isolation of an Hfr strain containing the sup15B mutation. For coarse mapping of the *sup15B* mutation, a new Hfr strain was isolated. The Hfr strain orginated from strain SB19, an F^+ strain carrying the *sup15B*, E31 mutations, and was isolated on the basis of efficient transfer of the suppressor mutation. This Hfr strain introduces the markers *ilv*, *metB*, and *argH* early and in that order. Preliminary attempts have failed to show the transfer of *xyl*, and it is possible that our Hfr strain is identical to strain Hfr6 (22) in origin and direction

TABLE 1. *List of bacterial strains used*

Strain	Source or reference[a]	Genotype[b]
AB1450	E. A. Adelberg (26)	*ilv-16, argH1, metB1, thi-2, his-1, gal-1, lac-8, mal-1, xyl-4, str, tsx, sup-am*, F⁻, λ⁻
AB2072	E. A. Adelberg	*thr-3, proA2, lac, galK2, trp-3, his-4, ara-9, malA1, mtl-1, metE46, ilv-95, thi, str, tsx-3, sup-am*, F⁻, λ⁻
AB2553	E. A. Adelberg	*ilvD188, metE46, thi, mtl-1, malA1, ara-9, his-4, trp-3, galK2, lac, proA2, str, ton-1, tsx-3, sup-am, supM*, F⁻, λ⁻
CH576	C. W. Hill, through J. Carbon	*argH, trpA36, ins, tsx, sup3, supA36*, F⁻, λ⁻
2320 (λ)	(16)	*lacZ2, thi, xyl, str, tsx*, F⁻, λ⁺
15B	(16)	*lacZ2, thi, xyl, str., tsx, sup15B*, F⁻, λ⁺
E31		Faster growing derivative of strain 15B; mutation determining faster growth rate is closely linked to *sup15B*
E54		Faster growing derivative of strain 15B; closely linked mutation
S30		Faster growing derivative of strain 15B; mutation determining faster growth is unlinked to *sup15B*
SB17		*trpC-oc, his-am, spc, tsx*, F⁻, λ⁺
SB19		*trpC-oc, his-am*, λ^r, *sup15B-E31*, F⁺, λ⁻
SB21		Hfr strain isolated from a culture of SB19
SB29		*metB, lacZ2, thi, xyl, str, tsx, sup15B-E31*, F⁻, λ⁺
SB38		*metB, lacZ2, trpA36, ins, tsx*, F⁻, λ⁺
SB42		*metB, lacZ2, trpA36, ins, tsx, supA36*, F⁻, λ⁺
SB68		Faster growing mutant of strain SB70; mutation determining faster growth is unlinked to *sup15B*
SB70		*lacZ2, trpA36, ins, tsx, sup15B, supA36*, F⁻, λ⁺
SB96		*argH, lacZ2, trpA36, ins, rif, tsx*, F⁻, λ⁺
SB100		*argH, lacZ2, thi, xyl, str, tsx*, F⁻, λ⁺

[a] Strains without source or reference listing were isolated in our laboratory.

[b] Abbreviations. Mutant allele names and state of episomes are given according to the convention proposed by Demerec et al. (11) and used by Taylor (31). Other abbreviations are as follows. λ^r, resistance to phage λ; *his*-am, *amber* (UAG) mutant codon present in a *his* gene; *trpC-oc*, *ochre* (UAA) mutant codon present in the *trpC* gene; *sup*-am, unidentified suppressor which confers the ability to translate amber (UAG) mutant codons; *ins*, mutation which restores normal growth in rich nutrient media to cells possessing *supA36* (7, 8).

of transfer.

The Hfr strain was isolated by using an appropriate modification of the method of sib selection of Cavalli-Sforza and Lederberg (9). The F⁺ strain (SB19) was *sup*⁺, his_{am}^-, trp_{oc}^-; the F-recipient (SB17) had the same *his*⁻ and *trp*⁻ mutations and was *sup*⁻ and *spc*-resistant; the two strains were otherwise isogenic. Several successive cycles of enrichment of the Hfr were used. The frequency of Hfr mutants capable of efficiently transferring the *ochre* suppressor could not be determined by direct plating of the replicate cultures at each cycle of enrichment; consequently, the following indirect test was used. From the culture with the highest concentration of Hfr cells in the previous cycle, several replicate subcultures were made; a variety of dilutions was used such that, for one of the dilutions, the concentration of Hfr cells would be about 0.1 Hfr cells per replicate subculture. The various subcultures were incubated overnight at 37 C. Each overnight culture was diluted 50-fold into 1 ml of L broth and was slowly rotated at 37 C for 2.5 hr. At the end of that time, 1 ml of a late-log culture of SB17 was added. The mixture of cells was rotated for 1 hr at 37 C; a 10-fold dilution was then made with saline, and both concentrations were spotted (about 0.01 ml) on a glucose-spectinomycin-minimal medium-agar plate which was subsequently incubated at 37 C. A subculture which was started from one of the higher dilutions and which showed a "jackpot" of Hfr cells by the previous test was chosen for the next cycle of enrichment. Appropriately higher dilutions were used to start the subcultures for this next cycle of enrichment. Six cycles of enrichment were sufficient to isolate a colony of the desired Hfr derivative.

Isolation of a spectinomycin-resistant mutant. The spectinomycin resistance mutation present in strain SB17 was obtained as follows. One drop of EMS was added to 5 ml of an overnight culture grown on T broth with slow rotation. The mixture was incubated for 30 min at 37 C, a 10-fold dilution into T broth was made, and the subculture was slowly rotated for 4 hr at 37 C. At the end of this period, a 10-fold dilution was made into T broth containing 100 μg of spectinomycin per ml and rotated slowly at 37 C. Two days later the culture was turbid; a sample of it was streaked on a T-spectinomycin plate incubated at 37 C, and one colony was isolated and purified.

Isolation of rifampin-resistant mutants. Rifampin-resistant mutants of spontaneous origin were isolated as follows. An overnight culture was plated on glucose-minimal medium plate containing the appropriate amino acid and vitamin supplements and 10 μg of rifampin per ml. The plates were incubated at 37 C. A mutant was chosen whose mutation to rifampin resistance was cotransducible with *argH* and which gave normal-size colonies in both minimal-rifampin and minimal plates.

Bacteriophage strains. Several *amber* and *ochre* mutants of phage T7 (17) were used to reveal the presence of the *ochre* suppressor. In addition, mutant S3 of phage T4 (27) was used to reveal the presence of the missense suppressor mutation *supA36*.

Culture media. Tryptone B_1 broth contained 10 g of tryptone (Difco), 5 g of NaCl, and 1 mg of thiamine per liter. Tryptone B_1 agar contained tryptone B_1 broth supplemented with either 6.5 g (soft agar), 10 g (1% agar) or 15 g (1.5% agar) of Difco agar per liter. Minimal medium mineral base contained

$KH_2PO_4 \cdot 3H_2O$, 9.2 g; KH_2PO_4 (anhydrous), 3 g; $(NH_4)_2SO_4$, 1 g; and $MgSO_4 \cdot 7H_2O$, 0.1 g. Carbon sources (glucose or lactose) were added to give a final concentration of 0.1%. Spectinomycin was dissolved in sterile water and added after the medium had been autoclaved to give a final concentration of 100 μg/ml. Rifampin was dissolved in dimethylsulfoxide at a concentration of 10 mg/ml and added to autoclaved medium to give a final concentration of 10 μg/ml. The biological activity of the rifampin in the plates decreases rapidly; consequently, only plates which had been prepared the same day were used. A fresh stock solution of rifampin was prepared every week and was kept at −20 C.

Transductions. Phage-mediated transductions were made using phage P1Kc according to the method of Luria et al. (24).

Interrupted mating experiments. Interrupted mating experiments were performed by the method of Hayes (19). Mating was interrupted by the addition of phage T6 at a multiplicity of infection of 50. Either streptomycin or spectinomycin was used to counterselect against the male donor.

Basis for selection of transductants possessing ochre and missense suppressors. Selection of transductants which had acquired the *ochre* suppressor mutation was based on the expression of the Lac$^+$ phenotype by recipients possessing the *ochre* mutation *lacZ2* obtained from strain 2320 (λ) (16). Selection of transductants which had acquired the missense suppressor mutation *supA36* was based on the expression of the Trp$^+$ phenotype of recipients possessing the mutation *trpA36* (5). This mutation, which is identical to *trpA23*, creates the codon AGA at that position in the messenger RNA for the *trp* operon which specifies the 210th amino acid residue of the tryptophan synthetase alpha chain (34).

Characterization of recombinant colonies. Recombinant clones obtained in the selective plates from conjugation or transduction were purified by streaking on the appropriate selective medium. Colonies from purified cells were picked into 0.5 ml of saline and spotted (about 0.01 ml per spot) either on minimal medium plates lacking the pertinent nutritional supplement or on supplemented minimal medium plates containing rifampin, or on both. Plates were incubated for 2 to 3 days at 37 C before scoring.

The presence of the *ochre* suppressor was detected as follows. A 1% tryptone B_1 plate was overlaid with 2 ml of molten 0.65% tryptone B_1 agar containing about 5×10^5 plaque-forming units (PFU) of the T7 mutant *oss18* or *oss30*. After the plate had solidified, the colony to be tested (suspended as described above) was spotted on the plate (about 0.01 ml per spot); 16 to 20 different colonies can be spotted on one plate. Two control plates were also spotted at the same time: one contained 5×10^5 PFU of wild-type T7, and the other contained no phage. (The plate containing wild-type T7 was useful in identifying P1-lysogenic transductants; these do not support the growth of wild-type T7.) The plates were incubated at 41 C overnight. A clone was scored as *su*$^+$ if a confluent "mini-lawn" formed on the no-phage plate but no lawn formed on the plates containing the *oss* mutant and wild-type T7, respectively. Conversely, if a lawn formed on both the *oss*- mutant plate and the no-phage plate but not on the T7+ plate, the clone was scored as *su*$^-$.

The presence of the *supA36* missense suppressor mutation was detected by a method analogous to that described above. Suspended cells from a colony were spotted on a set of three plates overlaid, respectively, with soft agar containing no phage, 5×10^6 PFU of mutant S3 of phage T4, and 5×10^6 PFU of wild-type T4. Plates were incubated overnight at 41 C and scored by the criteria analogous to those described in connection with the T7 nonsense mutants.

To avoid the slow growth rate caused by the *supA36* mutation in the haploid state (20), all the strains used as recipients of this mutation carried the mutation *ins* (7, 8). The *ins* mutation determines a species of glycine tRNA which can read the GGA codon, thus alleviating the severe lack of GGA-reading tRNA caused by the *supA36* mutation (8).

RESULTS

Localization of sup15B between argH and thi. In preliminary experiments using the F$^+$ strain SB19 as donor and AB2072 as a recipient, *su*$^+$ colonies were detected among the *ilv*$^+$ recombinants. The results of interrupted matings using the Hfr strain SB21 (*ilv*$^+$, *met*$^+$, *arg*$^+$, *su*$^+$) as donor and strain AB1450 (*ilv*$^-$ *metB*$^-$, *argH*$^-$, *su*$^-$) as recipient indicated that this Hfr strain introduces the *ochre* suppressor mutation about 30 sec later than it introduces *argH*.

Cotransduction of *sup15B*-E31 with *argH* and the orientation of the suppressor mutation relative to *metB* and *argH* were investigated by transduction with phage P1Kc. Strain E31 (*arg*$^+$, *met*$^+$, *su*$^+$) was used to grow a lysate of phage P1Kc. The recipient was strain AB1450 (*argH*$^-$, *metB*$^-$, and *su*$^-$). Transductants were selected for *arg*$^+$, or *met*$^+$, or both, and were scored for suppressor activity. The results (Table 2) demonstrate the order *metB-argH-su*. The occurrence of recombinants which are *met*$^-$, *su*$^+$ and *arg*$^-$, *su*$^+$, respectively, eliminates the possibility that either the *met*$^-$ or the *arg*$^-$ mutations are suppressible by the *ochre* suppressor, a possible source of confusion in mapping the suppressor.

The high frequency of cotransduction with *argH* suggested that the suppressor mutation is located between *argH* and *thi*. This question was investigated by transduction. Strains E31, E54, and S30 were used, respectively, as sources of lysates of P1Kc; all these strains are *met*$^+$, *su*$^+$, *thi*$^-$. Strain SB38 (*metB*$^-$, *su*$^-$, *thi*$^+$) was used as recipient. Transductants were selected for *su*$^+$ (suppressed *lac*$^+$ phenotype) and were scored for their *met* and *thi* phenotypes. The results (Table 3) imply the

order *metB-su-thi*. Taken together with the previous results, these results imply the order *metB-argH-su-thi*.

Possible identity of sup15B and supM. Eggertson (13, 14) has previously located an *ochre* suppressor (designated *supM*) in the same segment of the *E. coli* map (*argH-thi*) in which *sup15B* appears to map. This raised the possibility that the two suppressor mutations, even though of independent origin, may be identical. If they are not identical and, in particular, if they inserted different amino acids in response to the UAA and UAG codons, or the same amino acid but with different efficiency, then they might exhibit different patterns of suppression when tested with a variety of phage *ochre* and *amber* mutants.

To test whether *sup15B* and *supM* gave the same suppressor pattern, *supM* was first transduced into strain 2320 (λ), the strain in which the *sup15B* mutation arose. This was accomplished by using a lysate of P1Kc grown on strain AB2553, a *lac*$^-$ strain carrying the *supM* mutation, and selecting for the (suppressed) *lac*$^+$ phenotype. Nine of eleven *lac*$^+$ colonies thus obtained were *su*$^+$.

When the transductants carrying the *supM* mutation were streaked on tryptone B_1 plates and incubated overnight at 37 C, they showed the small colony size characteristic of strain 15B. All the *supM* transductants gave results identical to those obtained with strain 15B when both were spot-tested with a set of T7 *amber* and *ochre* mutants. This result is considered meaningful because the set of phage mutants was selected for sensitivity of growth response to differences in the amino acid inserted by the suppressor and to the efficiency of suppression. Although this approach cannot prove the identity of the two suppressors, the probability seems high that they are indeed identical.

Close linkage of sup15B to supA36. Hill et al. (20) have previously located the missense suppressor mutation *supA36* in the *argH-thi* segment of the *E. coli* map. We have performed crosses designed to investigate the linkage of *sup15B* to *supA36*.

The results of the first transduction suggested that probably the two suppressors are

TABLE 2. *Cotransduction of sup*$^+$ *with argH and metB and the order of the three markers*

Expt[a]	No. of colonies scored	Progeny genotypes[b]			No. of colonies[c]
		met	*arg*	*sup*	
1	61	*	D	D	18
		*	D	R	14
		*	R	D	1
		*	R	R	28
2	171	D	*	D	32
		D	*	R	32
		R	*	D	55
		R	*	R	52
3	34	*	*	D	22
		*	*	R	12

[a] In all experiments, the donor was strain E31 (*met*$^+$, *arg*$^+$, *sup*$^+$), and the recipient strain was AB1450 (*metB*$^-$, *argH*$^-$, *sup*$^-$). The order *metB*$^-$ *argH*$^-$ *sup* is the one which requires invoking the least number (1/266) of multiple integrational events.

[b] Asterisk indicates the selected marker in the transduction; D and R represent donor and recipient alleles, respectively, of the unselected genes.

[c] Less than 1% of the colonies scored could be spontaneous revertants, as determined by parallel controls.

TABLE 3. *Cotransduction of metB and thi with sup and the order of the three markers*

Expt	Donor Strain[a]	No. of colonies scored[b]	Progeny genotypes[c]			No. of colonies
			met	*sup*	*thi*	
1	S30	9	D	*	D	1
			D	*	R	2
			R	*	D	5
			R	*	R	1
2	E31	12	D	*	D	0
			D	*	R	2
			R	*	D	5
			R	*	R	5
3	E54	93	D	*	D	8
			D	*	R	13
			R	*	D	36
			R	*	R	36
Total			D	*	D	9
			D	*	R	17
			R	*	D	46
			R	*	R	42

[a] The same recipient, SB38 (*metB*$^-$, *sup*$^-$, *thi*$^+$), was used in every experiment. The three donors were *met*$^+$, *sup*$^+$, *thi*$^-$. No multiple integrational events need to be invoked if the order is assumed to be *metB-sup-thi*.

[b] Selection for *sup*$^+$ transductants was accomplished by selecting for the suppressed *lac*$^+$ phenotype. The acquisition of the suppressor was verified in every case by tests with T7 *amber* and *ochre* mutants. Total number of colonies scored: 114.

[c] Asterisk indicates the selected marker in the transduction; D and R represent donor and recipient alleles, respectively, of the unselected genes.

closely linked. Strain E31 (*arg*$^+$, *sup15B*-E31$^+$, *supA36*$^-$) was the source of a P1Kc lysate, and strain CH576 (*argH*$^-$, *sup15B*$^-$, *supA36*$^+$) was used as the recipient; transductants were selected for their *arg*$^+$ phenotype. Of 96 *arg*$^+$ transductants tested for their suppressor phenotypes, 55 had the combination of suppressor alleles of the donor and the remaining 41 had the recipient combination. No recombinants expressing either both or none of the suppressor alleles were found. Since both suppressors were unselected markers, the simplest interpretation of the results is that the two suppressor mutations are very closely linked.

A possible alternative interpretation of the above results is that the presence of one suppressor allele either epistatically prevents the expression of the other suppressor allele or results in the death of any cell in which the other suppressor allele is being expressed. To eliminate these alternatives, a more extensive attempt was made to isolate a recombinant possessing both suppressors. To accomplish this, strains were constructed which would permit applying selective pressure for the desired recombinant.

The following cross was performed to attempt to isolate a strain carrying both suppressor mutations. Strain S30 (*met*$^+$, *sup15B*$^+$, *supA36*$^-$) was used as a source of transducing lysate. Strain SB42 (*met*$^-$, *sup15B*$^-$, *supA36*$^+$) was the recipient; additionally, strain SB42 has the *ochre lac2* mutation, which permits selection for the expression of the *sup15B* mutation, and the missense mutation *trpA36*, which permits selection for the expression of the *supA36* mutation. Transductants were selected for the joint expression of both suppressor mutations (*lac*$^+$, *trp*$^+$ phenotype). Three clones were isolated and purified which showed the slow growth rate on tryptone B_1 plates and the nonsense phage suppressor pattern characteristically determined by the *sup15B* mutation, and also expressed the missense suppressor mutation as evidenced by their ability to suppress the missense mutant phage T4-S3. Two of the three clones, SB69 and SB99, were *met*$^-$, as was the recipient. In view of their rarity, the possibility cannot be excluded that SB69 and SB99 originated by de novo mutation rather than by recombination; this possibility is not inconsistent with the observed frequency of mutation of the *su*$^-$ strains used to *su*$^+$ strains which are indistinguishable from 15B. However, the third clone, SB70, is almost certainly a genuine recombinant, since it is *met*$^+$, as is the donor.

As expected, the two suppressor mutations present in strain SB70 (*sup15B* and *supA36*) are cotransducible with a frequency approaching 100%. The two suppressors were cotransduced in 204 out of 205 transductants selected for the transfer of either one of the two suppressors (Table 4). Furthermore, it is not excluded that the only possible recombinant clone could instead have originated as a consequence of the joint transfer of both suppressors, followed by reversion to the *sup15B*$^-$ phenotype and replacement of the colony due to the much faster growth rate of such revertants (16).

It is noteworthy that the same transduction which yielded strain SB70 also yielded strain SB49, a *met*$^+$ transductant which also expresses both suppressors. Strain SB49 differs, however, from strain SB70 in that the association between the two suppressors in the former strain is unstable. Two types of segregants are obtained if strain SB49 is cultured in the absence of selective pressure for the maintenance of both suppressors. One type of segregant is *sup15B*$^-$, *supA36*$^+$ and would be indistinguishable from its parental strain in the transduction were it not *met*$^+$. The other type of segregant is *met*$^+$, *sup15B*$^+$, *supA36*$^-$, the combination of alleles present in the other parental strain of SB49. Furthermore, unlike the case of strain SB70, the two suppressor

TABLE 4. *Cotransduction of sup15B and supA36 and their close linkage*

Expt[a]	No. of colonies scored[b]	Progeny genotypes[c]		No. of colonies
		sup15B	*supA36*	
1A	87	*	D	87
		*	R	0
1B	97	D	*	96
		R	*	1

[a] Recipient strain was SB38 (*metB*$^-$, *sup15B*$^-$, *supA36*$^-$). Donor strain was SB68 (*met*$^+$, *sup15B*$^+$, *supA36*$^+$). Strain SB68 is a spontaneous, fast-growing mutant of strain SB70; the mutation which confers faster growth is not cotransducible with the *sup15B* mutation (responsible for the slow growth of strain SB70).

[b] Selection for *sup15B*$^+$ was accomplished by selecting for the suppressed *lac*$^+$ phenotype. Selection for *supA36*$^+$ was accomplished by selecting for the suppressed *trp*$^+$ phenotype, made possible by the presence of mutation *trpA36* in strain SB38. The acquisition of the missense suppressor was verified in every transductant by tests with the T4 missense mutant S3.

[c] Asterisk indicates the selected marker in the transduction; D and R represent donor and recipient alleles, respectively, of the unselected genes.

mutations present in strain SB49 are seldom cotransduced, and the rare cotransductants observed invariably possess the unstable association of the suppressor mutations characteristic of strain SB49. It seems almost certain that strain SB49 has a tandem duplication which at the least includes the segment bounded by the two suppressor mutations. The occurrence of this type of chromosome abnormality in this region of the map (20) lends credence to our interpretation.

Localization of sup15B between argH and rif. Rifampin-resistant mutants map near *argH* (2, 3, 15, 23, 32) and can be cotransduced with high frequency with *supA36* (Squires and Carbon, *unpublished data*). This information, coupled with our finding of very close linkage of *sup15B* and *supA36*, suggested the possibility of ordering *sup15B* and *rif* with reference to *argH* by transduction.

Experiments were performed with two strains having mutations to rifampin resistance of independent origin. In one experiment, a spontaneous rifampin-resistant derivative of strain E31 (*met*$^+$, *arg*$^+$, *sup15B*-E31$^+$, *rif*^{r}) was used as the source of a transducing lysate. Strain AB1450 (*metB*$^-$ *argH*$^-$, *sup*$^-$, *rif*^{s}) was used as the recipient. Selection was for either *met*$^+$ (Table 5, experiment 1A) or *arg*$^+$ (Table 5, experiment 1B); transductants were scored for suppressor activity and for resistance to rifampin. In another experiment, strain SB68 (*arg*$^+$, *sup15B*$^+$, *supA36*$^+$, *rif*^{s}) was used as a source of transducing lysate. Strain SB96 (*argH*$^-$, *sup15B*$^-$, *supA36*$^-$, *rif*^{r}) was used as the recipient. Selection was either for *arg*$^+$ (Table 5, experiment 2A) or jointly for *sup15B*$^+$, *supA36*$^+$ (Table 5, experiment 2B). The results in Table 5 demonstrate the order *metB-argH-sup15B-rif*. It can also be inferred that *sup15B* and *rif* are very closely linked; among 168 *met*$^+$ or *arg*$^+$ transductants is experiments 1A, 1B, and 2A which received *sup15B* from the donor, 140 (83%) showed cotransduction of the donor marker with *sup15B*. Furthermore, among 96 *su*$^+$ transductants in experiment 2B (Table 5), 84 (88%) showed cotransduction of the *rif* marker of the donor. The much lower frequency of cotransduction of *thi* with *sup15B* (about 50%, see Table 3) implies the order *metB-argH-sup15B-rif-thi*.

The deoxyribonucleic acid (DNA)-dependent RNA polymerase activity of strain SB96 was tested for rifampin resistance by Parlane Reid (Dept. of Biochemistry, Univ. of Connecticut Medical School). At a rifampin concentration of 2 μg/ml, the RNA polymerase activity of a crude extract of strain SB96 is only 20% inhibited (with reference to the no-rifampin control), whereas that of strain SB38 (a *rif*^{s} strain isogenic with SB96) is 95% inhibited at the same concentration. Thus it seems likely that the *rif* locus mapped here is the structural gene for one of the RNA polymerase subunits.

Attempt to order sup15B and supA36 with respect to argH and rif. The finding that *sup15B* is closely flanked by *argH* and *rif* suggested the possibility of using these markers to order *sup15B* and *supA36* by selecting the class of integrational events which has one end in the interval between one of the suppressor mutations and *rif*. Strain SB68 (*arg*$^+$, *sup15B*$^+$, *supA36*$^+$, *rif*^{s}) was used as the source

TABLE 5. *Cotransduction of sup*$^+$ *and rif with argH and metB and their order*

Expt[a]	No. of colonies scored	Progeny phenotypes[b]				No. of colonies
		metB	*argH*	*sup*	*rif*	
1A	99	*	D	D	D	21
		*	D	D	R	3
		*	D	R	R	15
		*	R	R	R	60
1B	117	D	*	D	D	16
		D	*	D	R	4
		D	*	R	R	13
		R	*	D	D	59
		R	*	D	R	3
		R	*	R	R	22
2A	96		*	D	D	44
			*	D	R	18
			*	R	D	1
			*	R	R	33
2B	96		D	*	D	42
			D	*	R	10
			R	*	D	42
			R	*	R	2

[a] In experiments 1A and 1B, the donor strain was a rifampin-resistant mutant of strain E31 (*met*$^+$, *arg*$^+$, *sup*$^+$, *rif*^{r}); the recipient was strain AB1450 (*metB*$^-$, *argH*$^-$, *sup*$^-$, *rif*$^-$). In experiments 2A and 2B the donor strain was SB68 (*met*$^+$, *arg*$^+$, *sup15B*$^+$, *supA36*$^+$, *rif*^{s}); the recipient strain was SB96 (*met*$^+$, *argH*$^-$, *sup15B*, *supA36*$^-$, *rif*^{r}). The *rif*^{r} mutation in SB96 has been shown by Parlane Reid (*personal communication*) to determine rifampin-resistant polymerase activity. The order *metB - argH - sup - rif* is the one which requires invoking the least number of multiple integrational events.

[b] Asterisk indicates the selected marker in the transduction; D and R represent donor and recipient alleles, respectively, of the unselected genes. Selection for *sup*$^+$ in experiment 2B means joint selection for the transfer of *sup15B* and *supA36*, which are very closely linked.

of a transducing lysate. The recipient was strain SB96 (*argH*$^-$, *sup15B*$^-$, *supA36*$^-$, *rif*^{r}). The selection was for *arg*$^+$, *sup15B*$^+$ (or *supA36*$^+$), and *rif*^{r}. The results are shown in Table 6. Three cases of apparent recombination between the two suppressors were noted. Since the postulated recombinants are *arg*$^+$, it is virtually impossible that they could have originated by de novo mutation; thus in all likelihood they are genuine recombinants. The combined observations of the two crosses described in Table 6 suggest the order *argH-sup15B-supA36-rif:* The genotype of strain SB70 is also consistent with this order. Nevertheless, the small sample of genuine recombinants identified does not constitute a statistically reliable sample from which to deduce with certainty the order of the two suppressors.

DISCUSSION

Order of markers. The results of the mapping experiments reported above indicate the following order: *metB-argH-(sup15B, supA36)-rif-thi.* These results confirm previous findings that *supA36* maps between *argH* and *thi* (20), and that *rif*^{r} mutations are cotransducible with *argH* (2, 3, 15, 23, 32) and with *supA36* (Squires and Carbon, *personal communication*). In addition, our results indicate that *sup15B* is indistinguishable from *supM* (13, 14) with respect to growth rate, suppressor pattern, and map location and suggest that both suppressors arose through identical, though independent, mutations.

Close proximity of an ochre and missense suppressors. *Sup15B* and *supA36* are very close to one another; the frequency with which recombination between them occurs is of the order of 1%. The small number of cases in which genuine recombination between the two suppressor mutations was detected in this study disallows any inferences about the order of the two suppressors relative to outside markers.

TABLE 6. *Attempt to order sup15B and supA36 with respect to argH and rif*

Expt[a]	Selected markers	No. of colonies scored	Progeny phenotypes[b]		No. of colonies
			supA36	*sup15B*	
1A	*arg*$^+$, *sup15B*$^+$, *rif*^{r}	98	+	*	95
			−	*	3
1B	*arg*$^+$, *supA36*$^+$, *rif*^{r}	58	*	+	58
			*	−	0

[a] The donor strain was SB68 (*arg*$^+$, *sup15B*$^+$, *supA36*$^+$, *rif*^{s}). The recipient was strain SB96 (*argH*$^-$, *sup15B*$^-$, *supA36*$^-$, *rif*^{r}). Transductants were selected first for either *sup15B*$^+$ (suppressed *lac*$^+$ phenotype), in experiment 1A, or *supA36*$^+$ (suppressed *trp*$^+$ phenotype), in experiment 1B. The transductants were then replica-plated onto the appropriate triple selection plates (selection for *arg*$^+$, *rif*^{r}, and either *lac*$^+$ or *trp*$^+$, respectively).

[b] Asterisk indicates the selected marker.

The mutation to *supA36* is in the structural gene for a $tRNA^{Gly}_{GGA/G}$ (7, 21) and probably is a GC-to-AT transition at the base pairs position specifying the 3′ nucleotide of its anticodon; this altered tRNA can translate the AGA codon. The *sup15B* mutation is known to result in the appreance of an altered tRNA capable of translating the UAG codon (18). It is probable, though not yet demonstrated, that the *sup15B* mutation determined an anticodon alteration in a tRNA which in its wild-type form translates a sense codon. The close linkage between the two suppressor mutations raises the possibility that they might be mutations in the same structural gene. This possibility is made very unlikely by the isolation of strain SB70, which appears to be a genuine recombinant and which expresses both suppressor activities independently. Furthermore, the probable sequence of the anticodons of the two suppressor tRNA species makes it unlikely that the two mutations separately affect the two copies of a hypothetical microduplication of the same structural gene of the type reported by Russell et al. (30) for the structural gene for the minor species of $tRNA^{tyr}$. Finally, preliminary genetic and biochemical evidence obtained in our laboratory (*unpublished data*) suggests that the *sup15B* mutation determines an alteration of the major species (25) of tyrosine-accepting tRNA.

The previous arguments suggest that the two suppressor mutations altered the structural genes for two tRNA species which are functionally unrelated, in the sense that they do not appear to be members of the same "degenerate" set. The finding that the two mutations are closely linked raises an interesting possibility. Although the close linkage may be fortuitous, the possibility cannot be dismissed that the close linkage may have some functional significance. In particular, one may consider the possibility that the two structural genes belong to a segment of DNA which is transcribed as a unit; that is, the primary transcription product could be a single molecule of RNA which includes the sequence of both tRNA species and possibly others. Three other studies lend some plausibility to this proposal. Bernhardt and Darnell (4) have presented evidence that some mammalian tRNA species are generated by endonuclease action on RNA

molecules which are longer than the final tRNA, although the length of the extra piece(s) appears to be small (10 to 12 nucleotides). Secondly, experiments with the in vitro transcription of *E. coli* tyrosine tRNA I revealed a primary transcription product which is longer than the in vivo finished product (10); it is not yet clear, however, to what extent the extra length results from the inadvertent omission of some factors, analogous to the known sigma (6) and rho (29) factors, which might confer upon the core RNA polymerase the specificity required for specific initiation and termination of transcription of the tyrosine tRNA gene. Finally, Altman (1) reports the presence in vivo of a short-lived RNA which he interprets to be a precursor of tyrosine tRNA I because the former is stated to include the sequence of the latter (minus the CCA 3'-terminal trinucleotide) plus some 40 additional nucleotides.

Location of mutations E31 and E54. The preliminary mapping of *sup15B* was greatly facilitated by the availability of strain E31 because of the near normal growth rate of this strain. Cultures of 15B, on the other hand, frequently become replaced with faster growing derivatives, either sup^- or sup^+ (16; *unpublished results*).

Strains E31 and E54 were obtained by treatment of strain 15B with EMS; they were isolated as colonies larger than the 15B colonies on tryptone plates incubated at 37 C. The two mutant strains have a suppressor pattern different from that of 15B. A more detailed study of these mutants and their phenotypic properties will be reported elsewhere. It was previously shown that if phage P1Kc is grown on strain E31 (or E54) and a sup^- strain is transduced, selecting for the transfer of sup^+, the transductants obtained have the same suppressor pattern and growth rate as the E31 (or E54, respectively) donor, rather than that of strain 15B. These results were interpreted to indicate that both E31 and E54 have a second mutation very closely linked to the 15B mutation. An alternative which could not be ruled out by the other studies was the possibility that E31 (or E54) had sustained *two* MES-induced mutations, one being a reversion to the sup^- genotype and the second being an unrelated *ochre* suppressor mutation. This alternative explanation was considered unlikely because the E31-like mutations occurred no less frequently than reversions to the sup^- phenotype. The results obtained in this study (Tables 3 and 5) lead to the assignment of very similar, if not identical, locations to the *ochre* suppressor activity of strains E31, E54, and 15B, and consequently support the original interpretation. Thus it is probable that the new mutations in strains E31 and E54 are second mutations in the same tRNA structural gene assumed to be altered by the 15B mutation; a direct demonstration of this hypothesis awaits the positive identification of the species of tRNA affected by *sup15B* mutation.

Location of rifampin resistance mutations. Several reports have indicated a close linkage between rifampin resistance mutations and *argH* (2, 3, 15, 23, 32); however, there are no data published which distinguish on which side of *argH* *rif* is located. On the basis of a limited sample of data communicated by W. F. Doolittle, A. L. Taylor (31) tentatively assigned *rif* to a position counterclockwise to *argH*, between it and *metB*. By contrast, the mutations we have used map clockwise to *argH* (Table 5). The discrepancy appears to be based only on the fact that the numbers previously available were small. A. L. Taylor has kindly allowed us to examine W. F. Doolittle's data. We find that the two sets of data are congruent and, taken together, strongly support the order *metB-argH-rif*.

One of the rif^r mutations we have isolated and mapped (in strain SB96) has been shown by Parlane Reid to result in a DNA-dependent RNA polymerase activity which, when tested in crude extract, is significantly more resistant to rifampin than that of an isogenic rif^s strain. This result suggests that the rif^r mutation in strain SB96 is in a gene which specifies the primary structure of a subunit of the DNA-dependent RNA polymerase. The *rif* gene we have mapped may be close to, if not identical with, the *stv* gene, which is also known to map clockwise to *argH* (35); mutations at the *stv* gene also result in the synthesis of altered DNA-dependent RNA polymerase activity (resistant to streptovaricin) (35).

ACKNOWLEDGMENTS

We are grateful to P. Reid for RNA polymerase assays, to A. L. Taylor and J. Carbon for access to unpublished information on the location of rifampin resistance mutations, and to E. Adelberg, J. Carbon, and P. Reid for useful strains.

This work was supported by grant GB 13207 from the National Science Foundation.

LITERATURE CITED

1. Altman, S. 1971. Isolation of tyrosine tRNA precursor molecules. Nature N. Biol. **229**:19.
2. Austin, S., and J. Scaife. 1970. A new method for selecting RNA polymerase mutants. J. Mol. Biol. **49**: 263-267.
3. Babinet, C., and H. Condamine. 1968. Mutants résistants à la rifampicine, modifiés dans leur DNA-RNA-polymérase. C.R.H. Acad. Sci. (Paris) **267**:231-

232.
4. Bernhardt, D., and J. E. Darnell, Jr. 1969. tRNA synthesis in HeLa cells: a precursor to tRNA and the effects of methionine starvation on tRNA synthesis. J. Mol. Biol. **42**:43-56.
5. Brody, S., and C. Yanofsky. 1963. Suppressor gene alteration of protein primary structure. Proc. Nat. Acad. Sci. U.S.A. **50**:9-16.
6. Burgess, R. R., A. A. Travers, J. J. Dunn, and E. K. F. Bautz. 1969. Factors stimulating transcription by RNA polymerase. Nature (London) **221**:43-46.
7. Carbon, J., C. Squires, and C. W. Hill. 1969. Genetically altered $tRNA^{Gly}$ subspecies in *E. coli*. Cold Spring Harbor Symp. Quant. Biol. **34**:505-512.
8. Carbon, J., C. Squires, and C. W. Hill. 1970. Glycine transfer RNA of *Escherichia coli*. II. Impaired GGA-recognition in strains containing a genetically altered transfer RNA; reversal by a secondary suppressor mutation. J. Mol. Biol. **52**:571-584.
9. Cavalli-Sforza, L. L., and J. Lederberg. 1956. Isolation of preadaptive mutants in bacteria by sib selection. Genetics **41**:367-381.
10. Daniel, V., S. Sarid, J. S. Beckmann, and U. Z. Littauer. 1970. *In vitro* transcription of a transfer RNA gene. Proc. Nat. Acad. Sci. U.S.A. **66**:1260-1266.
11. 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.
12. di Mauro, E., L. Snyder, P. Marino, A. Lamberti, A. Coppo, and G. P. Tocchini-Valentini. 1969. Rifampicin sensitivity of the components of DNA-dependent RNA polymerase. Nature (London) **222**:533-537.
13. Eggertson, G. 1968. Mapping of *ochre* suppressors in *Escherichia coli*. Genet. Res. **11**:15-20.
14. Eggertson, G., and E. A. Adelberg. 1965. Map positions and specificities of suppressor mutations in *Escherichia coli* K-12. Genetics **52**: 319-340.
15. Ezequiel, D. H., and J. E. Hutchins. 1968. Mutations affecting RNA polymerase associated with rifampicin resistance in *Escherichia coli*. Nature (London) **220**: 276-277.
16. Gartner, T. K., and E. Orias. 1965. Pleiotropic effects of suppressors of a Lac- "operator negative" mutation in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. **53**:62-68.
17. Gartner, T. K., and E. Orias. 1966. Effects of mutations to streptomycin resistance on the rate of translation of mutant genetic information. J. Bacteriol. **91**:1021-1028.
18. Gartner, T. K., E. Orias, J. E. Lannan, J. Beeson, and P. J. Reid. 1969. The molecular basis of suppression in an ochre suppressor strain possessing altered ribosomes. Proc. Nat. Acad. Sci. U.S.A. **62**:946-951.
19. Hayes, W. 1957. The kinetics of the mating process in *Escherichia coli*. J. Gen. Microbiol. **16**:97-119.
20. Hill, C. W., J. Foulds, L. Soll, and P. Berg. 1969. Instability of a missense suppressor resulting from a duplication of genetic material. J. Mol. Biol. **39**:563-581.
21. Hill, C. W., C. Squires, and J. Carbon. 1970. Glycine transfer RNA of *Escherichia coli*. I. Structural genes for two glycine tRNA species. J. Mol. Biol. **52**:557-569.
22. Jacob, F., and E. L. Wollman. 1961. Sexuality and the genetics of bacteria. Academic Press Inc., New York.
23. Khesin, R. B., Z. M. Gorlenko, M. F. Shemyakin, S. L. Stvolinsky, S. Z. Mindlin, and T. S. Ilyina. 1969. Studies on the functions of the RNA polymerase components by means of mutations. Mol. Gen. Genet. **105**:243-261.
24. Luria, S. E., J. N. Adams, and P. C. Ting. 1960. Transduction of lactose utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348-390.
25. Nishimura, S., F. Harada, U. Narushima, and T. Seno. 1967. Purification of methionine-, valine-, phenylalanine- and tyrosine-specific tRNA from *Escherichia coli*. Biochim. Biophys. Acta **142**:133-148.
26. Pittard, J., and T. Ramakrishnan. 1964. Gene transfer by F' strains of *Escherichia coli*. IV. Effect of a chromosomal deletion on chromosome transfer. J. Bacteriol. **88**:367-373.
27. Reid, P., and P. Berg. 1968. T4 bacteriophage mutants suppressible by a missense suppressor which inserts glycine in place of arginine for the codon AGA. J. Virol. **2**:905-914.
28. Reid, P., T. K. Gartner, and E. Orias. 1969. Abnormal 30*S* ribosomal subunits determined by an *ochre* suppressor mutation in *Escherichia coli*. J. Bacteriol. **98**: 308-310.
29. Roberts, J. W. 1969. Termination factor for RNA synthesis. Nature (London) **224**:1168-1174.
30. Russell, R. L., J. N. Abelson, A. Landy, M. L. Gefter, S. Brenner, and J. D. Smith. 1970. Duplicate genes for tyrosine transfer RNA in *Escherichia coli*. J. Mol. Biol. **47**:1-13.
31. Taylor, A. L. 1970. Current linkage map of *Escherichia coli*. Bacteriol. Rev. **34**:155-175.
32. Tocchini-Valentini, G. P., P. Marino, and A. J. Colvill. 1968. Mutant of *E. coli* containing an altered DNA-dependent RNA polymerase. Nature (London) **220**: 275-276.
33. Webster, R. E., D. L. Engelhardt, N. D. Zinder, and W. Konigsberg. 1967. Amber mutants and chain termination *in vitro*. J. Mol. Biol. **29**:27-43.
34. Yanofsky, C., J. Ito, and V. Horn. 1966. Amino acid replacements and the genetic code. Cold Spring Harbor Symp. Quant. Biol. **31**:151-162.
35. Yura, T., and K. Igarashi. 1968. RNA polymerase mutants of *Escherichia coli*. I. Mutants resistant to streptovaricin. Proc. Nat. Acad. Sci. U.S.A. **61**:1313-1319.