JOURNAL OF BACTERIOLOGY, Jan. 1979, p. 433-439
0021-9193/79/01-0433/07$02.00/0

Vol. 137, No. 1

Characterization of Amber and Ochre Suppressors in *Salmonella typhimurium*

FRED WINSTON,[1]* DAVID BOTSTEIN,[1] AND JEFFREY H. MILLER[2]

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139,[1] and Département de Biologie Moléculaire, Université de Genève, Geneva, Switzerland[2]

Received for publication 5 July 1978

Amber and ochre suppressor mutations in *Salmonella typhimurium* were selected. The amino acid insertions directed by the suppressors were inferred from suppression patterns of *Escherichia coli lacI* amber mutations. These amber mutations only respond to nonsense suppressors that direct the insertion of particular amino acids. Four *Salmonella* amber suppressors characterized insert serine, glutamine, tyrosine, and (probably) leucine. Of the three ochre suppressors characterized, two direct the insertion of tyrosine and one directs that of lysine. Of the three amber and two ochre suppressors which have been mapped by phage P22 cotransduction, all are located in the same relative position on the *Salmonella* map as the analogous *E. coli* suppressors are on the *E. coli* map.

Nonsense suppression is the result of a mutation in a tRNA gene such that the altered tRNA reads a nonsense codon as sense. At some frequency, the altered tRNA inserts an amino acid at the nonsense codon, allowing elongation rather than termination of the polypeptide chain.

Most of the evidence for the role of tRNA in nonsense suppression comes from study of suppressor mutations in *Escherichia coli* (5, 6, 19). For those *E. coli* nonsense suppressors where the amino acid insertion has been elucidated, the determination has been carried out by chemical sequence analysis of a suppressed protein (5).

Miller and co-workers (1, 12) characterized a large series of *lacI* amber (Am) mutations and showed that a number of them make functional *lac* repressor only if a particular amino acid is inserted; suppressors inserting other amino acids do not result in synthesis of normally active repressor.

We selected several amber and ochre (Oc) suppressor mutations in *Salmonella typhimurium* and have genetically deduced the amino acid insertions they direct by analyzing their ability to suppress the various *lacI*(Am) mutations.

MATERIALS AND METHODS

Media. Liquid media used were LB broth (9) and M9 minimal medium and M9 supplemented with amino acids (M9-CAA) (17). Solid media used were LB plates, M9 plates, lambda plates (16), and MacConkey lactose and 5-bromo-4-chloro-3-indolyl-β,D-galactoside (XG) indicator plates (11). Semisolid medium was adapted from that described by Enomoto and Iino (2) and contained, per liter: 4 g of agar, 80 g of gelatin, 5 g of NaCl, 1 g of yeast extract, and 8 g of tryptone. Buffered saline was as described by Susskind et al. (18).

When necessary, minimal media were supplemented with amino acids at a concentration of 20 μg/ml and with histidinol at a concentration of 150 μg/ml. Tetracycline was used at a concentration of 25 μg/ml.

Chemicals. The sources of chemicals were as follows: tetracycline, Calbiochem; *o*-nitrophenyl-β,D-thiogalactoside, isopropyl-β,D-thiogalactopyranoside (IPTG), and rifampin, Sigma; XG, Bachem; streptomycin, Eli Lilly; and ethyl methane sulfonate (EMS), Eastman.

β-Galactosidase assays. β-Galactosidase assays and units are as described by Miller (11).

Bacterial strains. All bacterial strains are listed in Table 1, and all are F^-. All strain constructions were done by P22 transduction.

Strains of *S. typhimurium* LT2 carrying the *hisC527*(Am) allele were constructed by one of two methods. First, strain DB7000 was transduced to tetracycline resistance with a transducing lysate grown on the donor strain NK133, which carries a Tn*10* insertion in *hisD*, producing strain DB7126. We then transduced this strain to $hisD^+$ (selecting ability to grow on histidinol), using a transducing lysate grown on strain DB53. A transductant that was $hisD^+$ $hisC^-$ and tetracyline sensitive was isolated and called strain DB7136.

To construct strain DB6327 we transduced strain DB6306 to tetracycline resistance with a donor lysate grown on strain DB7279. A transducing lysate grown on this strain results in about 65% cotransduction of *hisC527* with *gnd114*::Tn*10*.

F' factors. All F' factors carrying the *lacI* alleles are listed in Table 2 and are described by Coulondre and Miller (1).

TABLE 1. *S. typhimurium LT2 and E. coli K-12 strains used*

Strain	Synonym	Genotype	Source or reference
S. typhimurium			
DB53	TR248	*cysA348*(Am) *hisC527*(Am)	J. Roth (7)
DB6174		*cysA1348*(Am) *hisC527*(Am) *proAB137*::Tn*10*	This paper
DB6241	TR166	*argF88 hisD2427*	J. Roth
DB6249		*cysA1348*(Am) *hisC527*(Am) *proAB137*::Tn*10 supD10*	This paper
DB6250		*cysA1348*(Am) *hisC527*(Am) *proAB137*::Tn*10 supE20*	This paper
DB6251		*cysA1348*(Am) *hisC527*(Am) *proAB137*::Tn*10 supF30*	This paper
DB6252		*cysA1348*(Am) *hisC527*(Am) *proAB137*::Tn*10 supJ60*[a]	This paper
DB6257		*argF88 hisD2427 rpoB*	This paper
DB6306	SJ374	*flaD42*	P. Hartman (3)
NK133		*hisD9426*::Tn*10 edd*$^-$	N. Kleckner
NK125		*trp125*::Tn*10 edd*$^-$	N. Kleckner
DB6327		*flaD42 hisC527*(Am) *gnd-114*::Tn*10*	This paper
DB7000		*leuA414*(Am)	D. Botstein
DB7001		*leuA414*(Am) *rpsL*	D. Botstein
DB7126		*leuA414*(Am) *hisD9426*::Tn*10*	This paper
DB7279		*leuA414*(Am) *hisC527*(Am) *gnd-114*::Tn*10*	This paper
DB7136		*leuA414*(Am) *hisC527*(Am)	This paper
DB7175		*leuA414*(Am) *hisC527*(Am) *argF88 rpoB*	This paper
DB7154		*leuA414*(Am) *hisC527*(Am) *supD10*	This paper
DB7155		*leuA414*(Am) *hisC527*(Am) *supE20*	This paper
DB7156		*leuA414*(Am) *hisC527*(Am) *supF30*	This paper
DB7157		*leuA414*(Am) *hisC527*(Am) *supJ60*[a]	This paper
DB7302		*leuA414*(Am) *hisC527*(Am) *supG50*	This paper
DB7303		*leuA414*(Am) *hisC527*(Am) *supC80*	This paper
DB7304		*leuA414*(Am) *hisC527*(Am) *tyrU90*	This paper
DB7401		*leuA414*(Am) *hisC527*(Am) *argF88 rpoB trp-125*::Tn*10*	This paper
DB7572		*leuA414*(Am) *cya-408*	D. Botstein
E. coli			
X7026r		Δ(*lac-pro*) *supE recA thi*	J. Miller (1)
XA101		*ara* Δ(*lac-pro*) *nalA metB araE*(Am) *rif supD*	J. Miller (1)
XA102		*ara* Δ(*lac-pro*) *nalA metB araE*(Am) *rif supE*	J. Miller (1)
XA103		*ara* Δ(*lac-pro*) *nalA metB araE*(Am) *rif tyrT*	J. Miller (1)
XA96		*ara* Δ(*lac-pro*) *nalA araE*(Am) *rif* Su6$^+$	J. Miller (1)

[a] *supJ* carries the designation *supH* in the current *Salmonella* genetic map (14). It has been renamed to distinguish it from *supH* of *E. coli*.

TABLE 2. *lacI(Am) mutations and their phenotypes in E. coli strains carrying various amber and ochre suppressor mutations*[a]

lacI allele	Amino acid codon affected	Amber suppressors				Ochre suppressors	
		supD	*supE*	*tyrT*	Su6$^+$	*supG*	*supC*
Wild type		+	+	+	+	+	+
Missense		−	−	−	−	−	−
Amber							
A8	Tyr47	−	−	+	−		
X7	Leu251	−	−	−	+		
A5	Gln18	−	+	−	−		
X1	Thr5	+	−	−	−		
A10	Ser61	+	+	s	s		
A25	Glu235?	s	+	s	s		
X13	Leu318	s	+	s	s		
A24	Trp220	s	s	s, +	s		
A20	Ser190, -191, or -193	+	s, +	s	s		
Ochre							
O22	Leu189	−	−	−	−	+	+
O8	Tyr47	−	−	−	−	−	+
O10	Gln55	−	−	−	−	−	−
O11	Gln78	−	−	−	−	s	+

[a] The Lac phenotypes are: +, normally inducible; −, constitutive; s, noninducible.

Bacteriophage strains. All phage P22 mutants are listed in Table 3.

Selection for spontaneous suppressor mutations. For selecting spontaneously arising suppressor mutations, five individual colonies of DB53 were each grown to stationary phase in M9-CAA at 37°C. The cells were washed and resuspended in the same volume of buffered saline, plated at various concentrations on M9 (glucose minimal) plates, and incubated at 37°C. Colonies which grew on these plates were purified twice and tested for the presence of suppressor mutations as described below.

Selection for EMS-induced suppressor mutations. The procedure for selection for EMS-induced suppressor mutations, originally described by Hilliker and Botstein (7), is the same as above with the following modifications: first, we used strain DB6174; second, each M9 plate was supplemented with 2 μg of the required amino acids per ml to allow a few rounds of growth after plating; and third, EMS on it was placed in the center of the plate.

Test for suppression of phage P22 amber mutants. Strains were tested for their ability to suppress various P22 amber mutants by streaking each P22 mutant (at a concentration of 10^8 phage per ml) on a lawn of each suppressor strain, and the plates were incubated at 37°C.

Episome transfers. For transfer of the F′ factors from their *E. coli* host (X7026r) to *Salmonella* (strain DB7001), 1 ml of the donor strain grown to approximately 2×10^8 cells per ml in LB was mixed with 5 ml of the recipient strain grown to approximately 5×10^8 cells per ml in LB in a 50-ml flask. The mixture was incubated overnight at 37°C, and dilutions were plated on the following day on M9 lactose plates containing leucine and streptomycin.

For transfer from *Salmonella* to *Salmonella*, donor and recipient strains were both grown in LB to around 2×10^8 cells per ml, and 0.05 ml of each was spread together on the proper selective medium. In both procedures transconjugants were purified once selectively and once permissively and then scored for their markers. In mating from DB7001 background into DB6174 background, transfer of F′ factors (which carry *lac* and *proAB*) was selected for by selecting *leu*$^+$ and *pro*$^+$ and then scoring for *lac*$^+$.

P22 transductions. Transducing lysates, made with P22*int*3 or a high-frequency transducing mutant, P22*int*3 HT12/4 (15), were prepared as described by Kleckner et al. (8). Transductions were carried out by mixing phage and cells at the desired multiplicity for 20 min at room temperature and then plating dilutions on the proper selective medium. For P22*int*3, transductions were done at a multiplicity of 10; for P22*int*3 HT12/4, transductions were done at a multiplicity of 0.1 to avoid double transduction events. Cotransductants were scored by replica plating or picking and stabbing with sterile toothpicks.

RESULTS

Basis for genetic determination of amino acid insertions by amber and ochre suppressor mutations. Miller et al. (2) and Coulondre and Miller (1) described several *lacI*(Am) mutations which make functional *lac* repressor only when a particular amino acid is inserted at the amber site. Amino acid insertions by the standard amber and ochre suppressors in *E. coli* have been determined by direct chemical analysis of suppressed proteins (5). We assumed in this work that each of the *lacI*(Am) mutations has the same suppression requirements in *Salmonella* as it does in *E. coli*. For example, a *lacI*(Am) mutation which makes functional *lac* repressor in *E. coli* only when a suppressor inserts glutamine was assumed to make functional *lac* repressor in *Salmonella* only when the *Salmonella* suppressor inserts glutamine. On this basis, we sought to determine the amino acid insertions directed by the *Salmonella* amber and ochre suppressor mutants simply by assaying β-galactosidase.

Selection of amber and ochre suppressor mutations. The selection of spontaneous amber and ochre suppressor mutations is based on the fact that a suppressor mutation resulting in phenotypic prototrophy in a double amber auxotroph will arise 10^5 to 10^6 times more frequently than independent reversion at both amber sites. The strain we used for suppressor selection (DB53) has two amber mutations resulting in auxotrophy for histidine and cysteine.

TABLE 3. *Suppression patterns of P22 amber mutations in Salmonella suppressor strains*

Strain	Suppressor mutation[a]	Suppression pattern									
		Wild type	1⁻*am*N18	1⁻*am*H21	1⁻*am*H58	8⁻*am*N26	13⁻*am*H101	12⁻*am*N11	3⁻*am*N6	23⁻*am*H79	23⁻*am*H316
DB7154	*supD*	+	+	+	+	+	+	+	+	+	+
DB7155	*supE*	+	+	−	−	−	+	+	+	+	+
DB7156	*supF*	+	+	+	+	+	−	+	−	+	+
DB7157	*supJ*	+	+	+	−	−	+	+	+	+	+
DB7302	*supG*	+	−	−	−	−	−	−	+	−	−
DB7303	*supC*	+	−	−	−	−	−	+	−	+	+
DB7304	*tyrU*	+	−	−	±	±	−	+	±	+	+

[a] Each strain is representative of a class of mutants having a given pattern of suppression. The nomenclature reflects the final outcome of experiments presented in the text.

As described in Materials and Methods, independent cultures of DB53 were grown to saturation, washed, and plated on M9 minimal agar plates at 37°C. After approximately 36 h, colonies began to appear. The plates were maintained at 37°C, and the time of appearance of each colony was noted. After 48 h, colonies were picked and purified two times, first on M9 plates and then on LB plates. Colonies were chosen for further analysis on the basis of time of appearance, size, and morphology.

Alternatively, suppressor mutations were selected after EMS mutagenesis by the method of Hilliker and Botstein (7). In this procedure, the strains used for mutagenesis carried F′ factors with distinguishing *lacI* alleles so that the selected suppressor mutation could be quickly screened for a specific amino acid insertion. After about 48 h, those colonies growing farthest from the center of the plate (to minimize the risk of picking up double mutants) were picked and purified as described before and also streaked out on XG plates to determine their suppressed *lacI* phenotype.

Characterization of suppressor mutations by their ability to suppress amber mutations in phage P22. Initially, suppressor mutations were distinguished phenotypically by their ability to suppress various P22 amber mutations. We hoped to distinguish amber and ochre suppressor mutations in this way, since in *E. coli* ochre suppressors are much less efficient than amber suppressors (5); we assumed the same to be true for *Salmonella*.

P22 gene products 1 and 8 are involved in head morphogenesis and gene product 13 is involved in cell lysis, whereas gene products 12, 3, and 23 are involved in DNA replication, DNA maturation, and regulation of the P22 late genes, respectively. In order to grow, the phage needs a greater amount of the gene products in the former group than in the latter group; this leads to the expectation that amber suppressors could suppress mutations in any of these genes whereas ochre suppressors could suppress only the mutations in genes 12, 3, and 23.

Seven different suppression patterns were found, showing that the suppressor mutations fall into seven qualitatively different sets. At least three isolates fell into each pattern with the exception of what we found to be *supJ*, for which we found only one representative. Three sets did not suppress (or suppressed poorly) mutations in genes 1, 8, and 13, confirming our expectation of two classes of suppressors (amber and ochre) in terms of apparent efficiency of suppression.

We then transduced representative suppressor mutations into strain DB7136, creating strains DB7154, DB7155, DB7156, DB7157, DB7302, DB7303, and DB7304. Their suppression pattern (Table 3) is the same as in the original background. We tentatively conclude that the mutations in DB7154, DB7155, DB7156, and DB7157 are amber suppressor mutations and those in DB7302, DB7303, and DB7304 are ochre suppressor mutations.

When suppression was tested at various temperatures, strain DB7157 was found to be somewhat cold sensitive (at 26°C) in its ability to suppress various P22 amber mutations (data not shown).

Characterization of suppressor mutations on XG indicator plates. A suppressor mutation in each phenotypic class was combined with each *lacI*(Am) mutation (carried on F′ factors) by mating in the appropriate F′ factor and then scored for the *lac* phenotype on XG plates. [For the EMS-induced suppressor strains, we first cured the strain of the resident F′ factor and then re-introduced the set of episomes carrying the *lacI*(Am) mutations.] Blue colonies on XG plates indicate high β-galactosidase levels and nonfunctional *lac* repressor. White colonies on XG plates indicate low β-galactosidase levels and functional *lac* repressor. By this technique, we were able to assay β-galactosidase levels quickly and qualitatively and make preliminary determinations of amino acid insertions directed by the various suppressor classes.

Characterization of suppressor mutations by direct β-galactosidase assay. To measure more quantitatively β-galactosidase activity in each suppressor-*lacI*(Am) combination, we performed β-galactosidase assays as described by Miller (11).

The first set of experiments (Table 4) used *lacI*(Am) mutations which result in nonfunctional *lac* repressor with any amino acid but one, as characterized by *E. coli* suppressor mutations (1). These results, when compared with the results for the characterized *E. coli* suppressors, strongly indicate that the *Salmonella* suppressor mutations we call *supD*, *supE*, *supF*, and *supJ* are inserting serine, glutamine, tyrosine, and leucine, respectively. The suppressors we call *supD*, *supE*, and *supF* are analogous to suppressors in *E. coli* called *supD*, *supE*, and *tyrT* (*supF*), whereas the suppressor designated *supJ* is analogous to the *E. coli* suppressor called $Su6^+$ (5). Although *supJ* is called *supH* in the current map of *Salmonella* (14), it has been designated *supJ* here to distinguish it from *supH* of *E. coli*.

Next, we assayed the induced β-galactosidase

levels in the suppressor strains when carrying a set of *lacI*(Am) mutations which in *E. coli* make functional *lac* repressor with particular amino acid insertions and make uninducible *lac* repressor (i^s phenotype) with other particular amino acid insertions, giving a unique pattern for each suppressor mutation. These results (Table 5) show that the *Salmonella* suppressor mutations, with the exception of the *supJ* mutation, closely parallel the *E. coli* suppressor mutations. For this set of strains, assays in the absence of IPTG gave only background levels of β-galactosidase, demonstrating sufficient suppresssion by the suppressor mutations to cause repression of β-galactosidase in the uninduced state.

To characterize the amino acid insertions directed by the ochre suppressor mutations, we used, in addition to the first set of *lacI*(Am) mutations, a set of *lacI*(Oc) mutations whose suppression patterns should uniquely identify tyrosine and lysine insertions. These results (Table 6) show that the suppression pattern of *Salmonella supG* strongly resembles that of *E. coli supG* (1). This demonstrates that the *supC* and *tyrU* suppressors insert tyrosine, whereas the *supG* suppressor mutation directs the insertion of lysine.

To distinguish more rigorously between amber and ochre suppressor mutations on a basis other than suppression of P22 amber mutations, we assayed β-galactosidase levels in all the suppressor strains carrying an F′ factor carrying a *lacI*(Oc) mutation, O22, characterized in *E. coli* to make functional *lac* repressor with any amino acid insertion. However, since only ochre suppressor mutations are able to suppress ochre mutations, one would expect only the *Salmonella* ochre suppressor strains to make functional *lac* repressor, resulting in low β-galactosidase levels. The results (Tables 4 and 5) indicate that *supD*, *supE*, *supF*, and *supJ* do not suppress the *lacI*(Oc) mutation and therefore are amber suppressor mutations, whereas *supC*, *tyrU*, and *supG* do suppress the *lacI*(Oc) mutation and therefore are ochre suppressor mutations.

TABLE 4. *Suppression by Salmonella amber suppressor mutations of lacI(Am) and lacI(Oc) mutations as measured by β-galactosidase assay*[a]

Suppressor mutation	*lacI*(Am) mutations[b]				*lacI*(Oc) mutation[b]
	A8	X7	A5	X1	O22
supD	83 (100)	9.8 (26)	49 (55)	1.8 (9.7)	81
supE	76 (103)	19 (47)	0.3 (0.6)	93 (100)	62
supF (*tyrT*)	0 (1)	44 (84)	40 (55)	85 (103)	78
supJ ($Su6^+$)	39 (101)	1.7 (2)	72 (93)	87 (80)	50

[a] F′ factors carrying five different *lacI* mutations were transferred into *Salmonella* strains DB6249, DB6250, DB6251, and DB6252 and *E. coli* strains XA101, XA102, XA103, and XA96 (see Table 1). β-Galactosidase assays were performed as described in the text. All values are normalized to 100 = the units of uninduced β-galactosidase for an F′ factor carrying a *lacI* missense mutation. The actual values for such a case ranged from approximately 125 to 250 units. The normalized values for the analogous *E. coli* suppressor mutations are in parentheses.

[b] The *lacI* alleles derive from amino acid codons in the wild-type gene as follows: A8, Tyr47 → amber; X7, Leu251 → amber; A5, Gln18 → amber; X1, Thr5 → amber; and O22, Leu178 → ochre.

TABLE 5. *Suppression by Salmonella amber suppressor mutations of lacI(Am) mutations as measured by β-galactosidase assay*[a]

Suppressor mutation	*lacI*(Am) mutations[b]				
	A20	A10	A24	A25	X13
supD	70 (86)	78	0.6 (0.1)	0.7 (0.1)	25 (39)
supE	5.8 (6.4)	106	1.0 (0.3)	71 (73)	40 (45)
supF (*tyrT*)	1.3 (0.2)	5.8 (2.6)	25 (35)	0.9 (0.1)	15 (14)
supJ ($Su6^+$)	6.1 (0.3)	35 (0.8)	1.3 (0.1)	23 (0.7)	19 (56)

[a] Procedures as described in footnote *a*, Table 4, except the strains were grown in medium containing 10^{-4} M IPTG. All values are normalized to 100 = the units of uninduced β-galactosidase for an F′ factor carrying a *lacI* missense mutation. The values for the analogous *E. coli* suppressor mutations are in parentheses.

[b] The *lacI* alleles derive from amino acid codons in the wild-type gene as follows: A20, Ser182 → amber; A10, Ser61 → amber; A24, Trp220 → amber; A25, Glu235(?) → amber; and X13, Leu318 → amber.

Mapping of the suppressor mutations. For mapping the suppressor mutations, we assumed each one to be in the same region of the map as its *E. coli* analog and then attempted to link it by P22 contransduction with known markers in that region.

We were able to show that five of the suppressor mutations are linked to known *Salmonella* markers by P22 cotransduction. The results of the cotransduction experiments for *supD*, *supF*, *supC*, *tyrU*, and *supJ* are shown in Table 7. The linkage of *supD* with *flaD* confirms the mapping previously done by Fankhauser and Hartman (3) on another isolate of *supD*. The map positions of *supF* and *supC*, slightly linked to *trp*, places them in the same region as two

TABLE 6. *Suppression by Salmonella ochre suppressor mutations of lacI(Am) and lacI(Oc) mutations as measured by β-galactosidase assay*[a]

Suppressor mutation	IPTG[c]	*lacI*(Am) mutations[b]				*lacI*(Oc) mutations[b]			
		A8	X7	A5	X1	O8	O10	O11	O22
supG	−	98	83	81	18	99	23	0.4	0.3
	+	−	−	−	−	106	95	1.0	
supC	−	0.4	64	65	75	0.5	71	1.0	0.3
	+	−	−	−	−	56	89	37	
tyrU	−	0.5	73	71	95				0.4

[a] Procedure as described in footnote *a*, Table 4. The F′ factors were mated into strains DB7302, DB7303, and DB7304 (see Table 1).

[b] The *lacI* alleles A8, X7, A5, X1, and O22 are described in Table 4. The last three derived from amino acid codons in the wild-type gene as follows: O8, Tyr47 → ochre; O10, Gly55 → ochre; and O11, Gln98 → ochre.

[c] +, Grown in 10^{-4} M IPTG; −, no inducer.

TABLE 7. *Mapping of suppressor mutations by P22 cotransduction*

Recipient strain	Marker selected	Marker scored	No. scored	No. of cotransductants	% Cotransduction
DB6327	*supD*	*flaD*	260	103	39.6
DB7401	*supF*	*trp*	1,282	22	1.7
DB7401	*trp*	*supF*	1,758	19	1.1
DB7401	*supC*	*trp*	254	6	2.4
DB7401	*trp*	*supC*	1,388	17	1.2
DB7175	*tyrU*	*argF*	1,036	152	14.7
DB7175	*argF*	*tyrU*	1,640	9	0.6
DB7572	*supJ*[a]	*cya*	104	104	100.0
DB7572	*cya*	*supJ*[a]	104	83	79.8

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

tyrosine-inserting suppressors in *E. coli*. Our mapping, however, merely links these two suppressor mutations to *trp* and does not place them in order with respect to any other markers in that region. These results differ from the results of Mojica-A (13), who reported that *supZ*, possibly the same suppressor mutation as our *supF*, although an independent isolate, was not linked to *trp* by phage P1 transduction, and suggest that *supZ* and *supF* may be different loci. Linkage of *tryU* to *argF* (first shown by J. R. Roth, personal communication) and *supJ* to *cya* likewise do not place these mutations in order with respect to markers in the region.

The lack of reciprocity in the cotransduction of *argF* and *tyrU* is probably due to the fact that strains carrying the *tyrU* suppressor mutation grow much more slowly than strains carrying the wild-type allele; therefore, there may be a selection for wild type at the *tyrU* locus when *argF* is selected in a transduction. This would alter the apparant cotransduction frequency.

The map position of *supJ* is in a region where at least two other tRNA genes (*supR* and *hisR*) are located (14).

Attempts to link *supE* with *gal* (less than 0.2% cotransduction) and *supG* with *nag* (less than 1.0% cotransduction) by P22 cotransduction failed.

DISCUSSION

A series of strains of *S. typhimurium* carrying amber and ochre suppressor mutations were characterized with respect to amino acids they insert in response to nonsense codons. Some of the suppressors were also genetically mapped. The genetic determination of the amino acid insertions is based on characteristic suppression patterns of well-characterized *E. coli lacI*(Am) and *lacI*(Oc) suppressor mutations. A close examination of β-galactosidase levels in the *Salmonella* suppressor strains shows, in most cases, striking quantitative agreement with the values found with the analogous *E. coli* suppressor strains.

The genetic locations of those *Salmonella* suppressor mutations which have been mapped is consistent with the assignment of amino acid insertions. The *E. coli* and *Salmonella* suppressor mutations which direct insertion of the same amino acids, in all cases where they were compared, are at the same relative location on their respective genetic maps. We anticipate that mapping of the remaining suppressor mutations will also place them in the same relative position as their *E. coli* analogs.

The one case where we question the certainty of the amino acid insertion is with *supJ*. Whereas the *Salmonella* β-galactosidase levels in Table 4 quite closely match those of *E. coli*, those in Table 5, for *supJ* suppression of the *lacI*(Am) mutations A25 and A10, do not. The higher β-galactosidase levels in *Salmonella* versus *E. coli* for these *supJ*-suppressed *lacI*(Am) mutations indicate that the suppressed *lac* repressor made in the *Salmonella supJ* strain is at least partially inducible by IPTG. The higher β-galactosidase levels cannot simply be ex-

plained by inefficient suppression of the *lacI*(Am) mutation by the *Salmonella* suppressor mutation (which would give a similar result) because the β-galactosidase levels in the uninduced cultures are at background level, indicating repression of β-galactosidase synthesis and therefore the presence of functional *lac* repressor.

The analog of *supJ* (called Su6$^+$) in *E. coli* is well characterized in *E. coli* as to its amino acid insertion (4) but is not well characterized genetically. It has been reported to lie within the region covered by F'14 of *E. coli* and also to be somewhat temperature sensitive (J. H. Miller, unpublished data). *Salmonella supJ*, closely linked to *cya*, lies in the analogous region in *Salmonella* covered by F'14 and is somewhat cold sensitive. The difference in the β-galactosidase results could be due to some difference in the physiology between *Salmonella* and *E. coli* or could reflect the insertion of an amino acid other than leucine.

We certainly do not believe that we have detected all the amber and ochre suppressor mutations possible in *Salmonella*; indeed, Michalka and Margolin (10) detected a large series of ochre suppressor mutations at a minimum of three different loci. Our selection required any amber or ochre suppressor mutation to be able to suppress the two auxotrophic mutations we used in our original selection scheme. Michalka and Margolin found that the nonsense mutation used to select nonsense suppressor mutations affects the spectrum of mutations detected.

For convenience and clarity, we have given the *Salmonella* suppressor mutations the same names as the analogous *E. coli* suppressor mutations except for *supJ*, for which the probable corresponding locus is not fully characterized. This reflects our belief that the corresponding mutations are the same between the two species and our hope that suppressor nomenclature will become more uniform in the future.

ACKNOWLEDGMENTS

We thank N. Kleckner, S. Gottesman, M. Susskind, J. R. Roth, and P. Hartman for helpful comments and bacterial strains. We also thank Pamela Oppenheimer for help in preparation of the manuscript.

This work was supported by Public Health Service grants GM-21253 and GM-18973 to D. B. from the National Institute of General Medical Sciences and by a grant from the Swiss National Fund (3.485.75) to J.H.M. F.W. was supported by Biophysics Training Grant NIH-5-T01-GM00778 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Coulondre, C., and J. H. Miller.** 1977. Genetic studies of the *lac* repressor repressor. III. Additional correlation of mutational sites with specific amino acid residues. J. Mol. Biol. **117**:525–575.
2. **Enomoto, M., and T. Iino.** 1963. Colonial dimorphisms in nonmotile *Salmonella*. J. Bacteriol. **86**:473–477.
3. **Fankhauser, D. B., and P. E. Hartman.** 1971. Direct selection for transduction of suppressor mutations and linkage of *supD* to *fla* genes in *Salmonella*. J. Bacteriol. **108**:1427–1430.
4. **Gopinathan, K. P., and A. Garen.** 1970. A leucyl-transfer RNA specified by the amber suppressor gene *Su6*$^+$. J. Mol. Biol. **47**:393–401.
5. **Gorini, L.** 1970. Informational suppression. Annu. Rev. Genet. **4**:107–134.
6. **Hartman, P. E., and J. R. Roth.** 1973. Mechanisms of suppression. Adv. Genet. **17**:1–105.
7. **Hilliker, S., and D. Botstein.** 1975. An early regulatory gene of *Salmonella* phage P22 analogous to gene *N* of coliphage λ. Virology **68**:510–524.
8. **Kleckner, N., R. K. Chan, B. K. Tye, and D. Botstein.** 1975. Mutagenesis by insertion of a drug-resistance element carrying an inverted repetition. J. Mol. Biol. **97**:561–575.
9. **Levine, M.** 1957. Mutations in the temperature phage P22 and lysogeny in *Salmonella*. Virology **3**:22–41.
10. **Michalka, I., and P. Margolin.** 1977. Ochre suppression in *Salmonella typhimurium*. Genetics **86**:237–260.
11. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Miller, J. H., D. Ganem, P. Lu, and A. Schmitz.** 1977. Genetic studies of the *lac* repressor. I. Correlation of mutational sites with specific amino acid residues: construction of a colinear gene-protein map. J. Mol. Biol. **109**:275–301.
13. **Mojica-A, T.** 1975. Transduction by phage P1CM clr-100 in *Salmonella typhimurium*. Mol. Gen. Genet. **138**: 113–126.
14. **Sanderson, K. E., and P. E. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471–519.
15. **Schmeiger, H.** 1972. Phage P22 mutants with increased or decreased transduction abilities. Mol. Gen. Genet. **119**:75–88.
16. **Signer, E. R., and J. Weil.** 1968. Recombination in bacteriophage I. Mutants deficient in general recombination. J. Mol. Biol. **34**:261–271.
17. **Smith, H. O., and M. Levine.** 1964. Gene order in prophage P22. Virology **27**:229–231.
18. **Susskind, M., A. Wright, and D. Botstein.** 1971. Superinfection exclusion by P22 prophage in lysogens of *Salmonella typhimurium*. II. Genetic evidence for two exclusion systems. Virology **45**:638–652.
19. **Whitfield, H.** 1972. Suppression of nonsense, frameshift, and missense mutations, p. 243–283. *In* L. Bosch (ed.), The mechanism of protein synthesis and its regulation. North-Holland, Amsterdam.