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Suppression of Amber and Ochre Mutants in *Salmonella typhimurium* by a Mutant F'-1-*gal* Factor Carrying an Ochre Suppressor Gene

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A *Salmonella typhimurium* strain was given the amber mutation *hisC527* by transduction, made galactose-negative by mutation, then infected with the F'-1-*gal* factor. Of 107 spontaneous and mutagen-induced histidine-independent mutants tested, 3 proved to result from suppressor mutations within the F' factor. The mutant F' factors, when transferred to *S. typhimurium* and *E. coli* auxotrophs, suppressed amber and ochre but not UGA or missense mutants, and are inferred to carry ochre suppressor genes. Attempts to isolate an F' amber suppressor mutant were unsuccessful. A suppressor F' factor was transferred to 14 rough mutants which had been isolated from LT2 *hisC527* (amber) by selection for resistance to phage P22.c2. One rough mutant was partly suppressed, as shown by its acquisition of O agglutinability and by alterations in its phage resistance pattern. Phage P22h grown on the suppressed mutant cotransduced its *rf.* gene with *cysE*$^+$ and with *pyrE*$^+$, and the affected locus is inferred to be *rfaL*. Both the original and the mutant F' factors conferred resistance to the rough-specific phage Br60, which is therefore "female-specific."

There is sometimes occasion to introduce a known amber suppressor or ochre suppressor gene into a given strain of *Salmonella typhimurium*, for instance to test whether a particular mutation is of one of these suppressible nonsense classes. The most generally available method, namely, transduction, is sometimes inconvenient. For instance, if the suppressor-carrying strain to be used as a donor has the wild-type allele of the nutritional character in the recipient known (or suspected) to be suppressible, then the transductants acquiring the wild-type nutritional gene may outnumber those acquiring the suppressor gene when selection is made for prototrophy. F' factors provide a convenient method for the easy introduction of a short piece of bacterial chromosome of defined composition, and the F'-1-*gal*$^+$ factor of *Escherichia coli* is known to include a locus or loci which can mutate to nonsense suppressor (11). We here describe the isolation in an *S. typhimurium* host of a mutant F'-1-*gal*$^+$ factor carrying an ochre suppressor. This operation was facilitated by the availability of many *S. typhimurium* auxotrophic mutants of known amber or ochre character (1, 16). The suppressor-bearing F'-1 factors described in *E. coli* were unstable (11), apparently because of frequent recombination of the episomal and chromosomal regions carrying the suppressor; no instability of this sort was observed in the case of our factor, presumably because recombination was prevented by the poor homology of the episomal genes of *E. coli* origin with their chromosomal homologues of *S. typhimurium* origin.

We chose as bacterial host a genetically marked derivative of a wild-type *S. typhimurium* strain, M7471, of the subtype (termed "FIRN") characterized by absence of fimbriae and failure to ferment inositol and rhamnose (8). We had first to introduce an amber auxotrophic mutation, to permit detection of the presence of a suppressor, and to obtain a galactose-negative mutation, so that introduction of the F'-*gal*$^+$ factor could be recognized.

We used the suppressor-bearing plasmid to test the suppressibility of a series of nonsmooth (*rf.*) mutants of LT2, and describe the properties

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of a suppressible mutant shown to be of class *rfa*.

MATERIALS AND METHODS

Media. Oxoid nutrient extract broth no. 2 (CM 67) and blood-agar base (CM 55) were used. Galactose fermentation was tested on Difco eosin-methylene-blue base agar with galactose (1%). The defined medium was Davis minimal, with or without agar, and with glycerol (0.2%) instead of glucose as carbon source; citrate was omitted for tests on ability to utilize galactose. Required amino acids were added at 20 μg/ml. Cultures were incubated at 37 C, liquid cultures being grown without shaking.

Bacterial strains and phages. The strains used are listed in Table 1. TV242, the genetically marked subline of M7471, had been prepared by T. V. Sub-

TABLE 1. *Bacterial strains*

Strain no.	Source and reference	Genotype[a]
Starting strains		
TV242	T. V. Subbaiah (Ph.D. Thesis, Univ. of London, 1963)	M7471 (*colEl-30*) *leu-1051 malB479 cysI1173*
	P. Hartman (16)	LT2 *hisC527* (amber)
TR112	J. Roth	K12 gal_1 gal_4 *met lac T1-r T3-r str* (F'-1-*gal*$^+$)
SL699	(Stocker collection)	LT2 *metA22 trpB2 H1-b H2-enx* "cured of a B phage" (*colE1-30*) *azi galK411*
SR315	N. Zinder, via K. Sanderson (9)	LT2 *leu gal*, male (F' or Hfr), transfers clockwise from *cysB*
SL1547	K. Sanderson (10)	LT7 *cysE30 gal*
	K. Sanderson (10)	LT2 *pyrE125*
Derived strains		
SL4522	TV242 given *hisC527* by transduction from LT2 *hisC527* (amber)	M7471 (*colE1*) *leu malB cysI his*(amber)
SL4525	Galactose-epimerase mutant of SL4522 obtained by phage selection	M7471 (*colE1*) *leu malB cysI his*(amber) *gal-459*
SL4527	SL4525 given F'-1-*gal*$^+$ by contact with TR112	M7471 (*colE1*) *leu malB cysI his*(amber) *gal* (F'-1-*gal*$^+$)
SL4528	Spontaneous histidine-non-requiring mutant of SL4527	M7471 (*colE1*) *leu malB cysI his*(amber) *gal* (F'-1-*gal*$^+$ *sup-812*)
SL4639	Suppressible "rough-sensitive" P22-resistant mutant of LT2 *hisC527* (amber)	LT2 *hisC527* (amber) *rf.-690*
SL4666	SL4639 given F'-1-*gal*$^+$ *sup-812* by contact with SL4528	LT2 *hisC527* (amber) *rf.-690* (F'-1-*gal*$^+$ *sup-812*)
Monoauxotrophic tester strains, of known codon character	J. Roth (16)	LT2 *hisC508*(amber)
	J. Roth (16)	LT2 *hisC869*(amber)
	J. Roth (16)	LT2 *hisC342*(ochre)
	J. Roth (16)	LT2 *hisC354*(ochre)
	J. Roth (16)	LT2 *hisC502*(ochre)
	J. Roth (16)	LT2 *hisC514*(ochre)
	J. Roth (16)	LT2 *hisG200*(UGA)
	J. Roth (16)	LT2 *hisG611*(UGA)
C9870	C. Yanofsky (19)	K12 *trpC9870*(amber)
C9875	C. Yanofsky (19)	K12 *trpC9875*(ochre)
D9929	C. Yanofsky (19)	K12 *trpD9929*(missense)
AE1	C. Yanofsky (19)	K12 *trpA-E*(deletion)
Suppressor-carrying control strains		
TA131	J. Roth (16)	LT2 *hisC527*(amber) *sup-501*(amber suppressor)
TA138	J. Roth (16)	LT2 *hisC527*(amber) *sup-508* (amber suppressor)
TA149	J. Roth	LT2 *hisC342*(ochre) *sup-519*(ochre suppressor)

[a] LT2, LT7 and M7471 are *S. typhimurium*, K-12 is *E. coli*. M7471 is of the "FIRN" wild type, which differs from LT2 wild type by absence of fimbriae and failure to ferment rhamnose and inositol (8). Gene symbols curtailed except at first mention.

baiah and B. A. D. Stocker (*unpublished data*) by introduction of a colicine factor, followed by three successive exposures to ethyl methane sulfonate. P22.L4, a nonlysogenizing variant of P22 (12), was used for transduction. For the phages used for differentiation of nonsmooth mutants, see Gemski and Stocker (5) and Wilkinson and Stocker (17). Phage SP6 is a "female-specific" phage (N. D. Zinder, *personal communication*) active on strain LT2, smooth or rough, but not on derivatives carrying the F factor of *E. coli* strain K-12. Amber mutants of phage P22 were received from R. A. Kolstad (7).

Transduction. To introduce the known amber auxotrophic gene *hisC527* into strain TV242, a young culture of TV242 in defined medium was mixed with P22.L4 grown on LT2 *hisC527* to give a multiplicity of ca. 50. After 20 min at room temperature, the cells were washed, resuspended in defined medium with cystine and leucine, and incubated at 37 C for 90 min to permit phenotypic expression of *his*$^-$ transductants and exhaustion of their content of free histidine. A penicillin enrichment was then applied (6). The cells were washed and suspended in defined medium with cystine and leucine and with sucrose (10%) and magnesium sulfate (0.01 M); after 2 hr at 37 C, penicillin G (4,000 units/ml) was added. After 90 min, penicillinase was added, and samples were diluted in saline (so as to rupture spheroplasts) and plated on defined agar with cystine and leucine and a concentration of histidine (2 μg/ml) such that histidine-requiring cells produced very small colonies. After 48 hr, all minute colonies were picked and tested for nutritional character.

To test the cotransducibility of the suppressible *rf.* mutation, phage P22h was grown on the suppressor-bearing derivative and used to evoke *cys*$^+$ transductants from SL1547 (= LT7 *cysE30 gal*) and *pyr*$^+$ transductants from LT2 *pyrE125*. Plates of selective medium were flooded with cultures of the recipient strains and allowed to dry, and drops of lysate (titer ca. 2×10^9/ml) were applied. Non-exacting transductants were purified and tested for rough character by tests of phage sensitivity.

Phage methods. Phages were propagated by the soft-agar-layer method. Phage sensitivities were determined by depositing drops (titers ca. 10^8/ml) on surface-inoculated plates of nutrient agar or nutrient agar supplemented with galactose (0.5%). Phage-resistant mutants were obtained by picking from colonies appearing in areas of confluent lysis. After purification they were characterized by tests of phage sensitivity, galactose fermentation, and galactose utilization and by slide-agglutination tests with anti-O sera (obtained from the Communicable Diseases Center, Atlanta, Ga.), diluted in 0.2% NaCl to avoid nonspecific agglutination.

Other genetic methods. Histidine-independent mutants, spontaneous or mutagen-induced, were obtained from surface-inoculated plates of defined medium lacking histidine, with filter-paper discs soaked in ethyl methane sulfonate or saturated aqueous solution of nitrosoguanidine. Conjugational crosses, for transfer of F′ factors, were made by incubation of mixed broth cultures, overnight for *S. typhimurium* × *E. coli* or 2 hr for *S. typhimurium* × *S. typhimurium* crosses.

RESULTS

Introduction of hisC527 by transduction. The *his*$^-$ gene of LT2 *hisC527*, a known amber allele (16), was transduced by P22.L4 into TV242, i.e., *S. typhimurium* M7471 (*colE1*) *leu malB cysI*. Two hundred small colonies were picked from approximately 5,000 colonies produced by survivors of the penicillin treatment of the transduction mixture. Three failed to grow on defined medium supplemented with cystine and leucine and one of these three responded to histidine. It was inferred to be a *hisC527* transductant, since it gave no histidine-independent colonies on treatment with P22.L4 grown on LT2 *hisC527* but many when the donor was either *his*$^+$ or LT2 *hisC527* carrying a suppressor of amber. This *his*$^-$ clone, SL4522, retained the characters of TV242. We shall here call it TV242 *his*(amber).

Isolation of galactose-negative mutant from TV242 (amber). Two of 20 spontaneous phage-resistant mutants, isolated from TV242 *his* (amber) by selection with a mixture of phages P22.c2 and Felix 0, failed to ferment galactose and were sensitive to phage C21. One of them, SL4525, had the phage-resistance pattern typical of mutants lacking uridine diphosphate (UDP)-galactose-4-epimerase (17). It regained sensitivity to smooth-specific phages when tested on nutrient agar supplemented with glucose and galactose (Table 2); this indicates deficiency of galactose-epimerase rather than of UDP-glucose pyrophosphorylase (17). SL4525 was therefore assigned the mutation number *gal-459*; it will here be called TV242 *his*(amber) *gal*. (This mutant was not sensitive to galactose and it remained partly sensitive to rough-specific phages even on medium supplemented with glucose and galactose, observations which suggest that it may be partly deficient in one or both of the other two enzymes of the galactose pathway, rather than deficient only in the epimerase.)

Transfer of F′-1-gal$^+$ into TV242 his(amber) gal. The F′-*gal*$^+$-*att*λ factor carried by strain TR112, a K-12 *met lac gal* strain, was transferred into TV242 *his*(amber) *gal* by overnight mixed culture. A clone, SL4527, obtained by selection for galactose-positive methionine-positive phenotype, had the phage-sensitivity pattern of smooth *S. typhimurium* (Table 2). It was inferred to be TV242 *his*(amber) *gal* carrying F′-1-*gal*$^+$. This inferred constitution was confirmed by its production of galactose-nonfermenting segregants

TABLE 2. *Phage sensitivites of mutants and F' derivatives*

Strain		Medium[a]	Phages[b]								
			Smooth-specific	Smooth- and rough-specific		Rough-specific					
No.	Description		P22, P22h, and 9NA	FO	SP6	6SR	Br60	Ffm	C21	Br2	P221
SL4522	Smooth, *gal*$^+$	Nutr	+	+	+	−	−	−	−	−	−
SL4525	UDP galactose-epimerase negative	Nutr	−	−	+	−	+	+	+	±	+
SL4525	UDP galactose-epimerase negative	Nutr + galactose	+	+	+	−	±	±	±	±	±
SL4527	SL4525 given F'-1-*gal*$^+$	Nutr	+	+	−	−	−	−	−	−	−
hisC527	Smooth	Nutr	+	+	+	−	−	−	−	−	−
SL4639	*rfa-690* mutant	Nutr	−	+	+	+	+	+	−	+	+
SL4666	*rfa-690* (F'-1-*sup-812*)	Nutr	+	+	±	±	−	±	−	±	+

[a] Media used were Oxoid blood-agar base (Nutr) with or without added galactose (0.5%) as indicated.
[b] Plates were surface-inoculated and spotted with 0.01 ml of phage lysate (ca. 10^8 plaque-forming units/ml). The plates were incubated at 37 C and examined at 18 hr. Symbols: +, confluent lysis; −, no lysis; ±, semiconfluent or faint lysis.

and by its ability to transfer the *gal*$^+$ character by mixed culture to an LT2 *met trp gal* tester strain, SL869.

Isolation of mutants with nonsense suppressor mutation in F' factor. A strain which is auxotrophic by a nonsense mutation yields non-exacting mutants by mutation of the nonsense codon and by nonsense suppressor mutation at any of several chromosomal loci. In a strain carrying an appropriate F'-factor suppressor, mutations might occur also in episomal loci, and clones containing an episomal suppressor could presumably be identified by transferring the episome to a suitable recipient. Spontaneous histidine-independent mutants isolated from TV242 *his*(amber) *gal* (F'-1-*gal*$^+$) were grown in mixed culture with LT2 *hisC527*(amber). Samples were plated on minimal agar, on which neither the *leu cys* donor nor the *his* recipient could grow, but on which recipient cells which had obtained an F' factor carrying an amber or ochre suppressor would be expected to grow. One of the 24 mating mixtures gave ca. 500 colonies from a 0.1-ml inoculum. We inferred that the effective histidine-independent mutant, SL4528, had a nonsense suppressor, which we designate *sup-812*, in its F'-1-*gal*$^+$ factor. This conclusion was supported by the observation that the galactose-negative segregants it produced were histidine-exacting. The mutant F' factor was transmitted to an intermediate LT2 galactose-negative strain, SL699, and from it into a galactose-negative and histidine-exacting segregant, which, as expected, then became histidine-non-exacting as well as galactose-positive.

Specificity and efficiency of the F'-borne suppressor. Strains SL4527 and SL4528, i.e., TV242 *his*(amber) *gal* carrying either the original F'-1-*gal*$^+$ factor or its suppressing derivative, were mated with various monoauxotrophic *S. typhimurium* and *E. coli* mutants (Table 1). No non-exacting colonies were obtained in crosses in which the donor carried the original form of the plasmid, but when it carried the mutant plasmid, non-exacting colonies were obtained from three LT2 *his*(amber) and five LT2 *his*(ochre) mutants and from *E. coli* K-12 *trp*(amber) and *trp*(ochre) mutants. No colonies were obtained in crosses with two LT2 *his* mutants of the UGA nonsense class or with *E. coli* missense and deletion *trp* mutants. These results indicate that the plasmid-borne suppressor *sup-812* is an ochre suppressor, since it suppresses both amber and ochre.

As the galactose-epimerase defect of TV242 *his*(amber) *gal* is repaired by the F'-*gal* factor, it is phenotypically smooth and fully sensitive to phage P22. The derivative strain carrying the suppressor plasmid was therefore tested for sensitivity to amber mutants of phage P22. Control strains TA131 and TA138 carrying the known amber suppressors *sup-501* and *sup-508* were lysed by all of three P22 amber mutants (7). TV242 *his*(amber) *gal* (F'-*gal*$^+$*sup-812*) and a control strain TA149, carrying the known ochre

suppressor *sup-519*, were almost or entirely resistant to them, i.e., the known and the suspected ochre suppressors both failed to effectively suppress the amber mutations in P22. Similarly, observations on growth on defined medium without histidine indicated that *sup-812* and a known ochre suppressor suppressed *his*(amber) mutations less effectively than dia a known amber suppressor—the diameter of discrete colonies after 2 days being < 1 mm for *sup-812* and the ochre suppressor and ca. 1.5 mm for the amber suppressor (and ca. 2.2 mm on histidine-supplemented medium).

Isolation of further suppressor mutants. The test used to detect the episome-borne *sup-812* was made on a further 31 spontaneous and on 52 nitrosoguanidine-induced histidine-independent mutants. Two of these 83 cultures transmitted a suppressor to LT2 *hisC527*. Both of these episome-borne suppressors, *sup-813* and *sup-814*, suppressed both ochre and amber LT2 *hisC* mutants, and we infer them to be ochre suppressors, perhaps identical to *sup-812*. In a further attempt to obtain an episomal amber suppressor, additional histidine-independent mutants were isolated and tested for sensitivity to a P22 amber phage stock. Seven of 36 spontaneous and 54 of 60 ethyl methane sulfonate-induced mutants were sensitive, which indicated that they carried amber suppressors. To test whether any of these 16 amber suppressors were plasmid-borne, a galactose-negative segregant of each was picked from an EMB-galactose medium inoculated from a broth culture (about 1% of the colonies were galactose-negative or sectored). All 61 purified galactose-negative segregants were histidine-independent. The continued suppression of the amber *his* mutation indicated that all the suppressors were chromosomal, since they were retained when the plasmid was lost. Further attempts to obtain an F′-*1-gal*$^+$ factor carrying an amber suppressor by various other methods were also unsuccessful.

Isolation of rough mutants and test of effect of sup-812 on them. Strain LT2 *hisC527*(amber) was exposed to phage P22.c2, and 16 spontaneous nonsmooth mutants were isolated. When tested with a set of phages which distinguish various classes of nonsmooth mutants, 14 gave the pattern, termed "rough-sensitive," characteristic of *rfb* mutants and of one class of *rfa* mutant (Table 2, pattern of strain SL4639; reference 5). All these 14 mutants lacked O antigen factor 4, as determined by slide agglutination tests. Each was mated with SL4528, i.e., the cystine- and leucine-requiring strain TV242 *his*(amber) carrying the suppressing F′ factor. The mixtures were plated on defined medium to select recipient clones which had become histidine-independent by acquisition of the suppressor-carrying F′ factor. After purification the suppressor-carrying derivatives of the rough mutants were tested for phage sensitivities and for O agglutinability, properties which were found of value in detecting suppression of rough mutations (T. Kuo, Ph.D. Thesis, Stanford Univ., Stanford, Calif., 1969). All the derivatives were resistant or partly resistant to the rough-specific phage Br60, which attacked the parent rough strains not carrying the F′ factor. They were also partly resistant to the "female-specific" phage SP6, which attacks both smooth and rough LT2 F$^-$ lines, but not their F$^+$ and Hfr derivatives (N. Zinder, *personal communication*). We assume that diminished sensitivity to phage Br60 likewise results from the presence of the F factor itself, not from the action of the suppressor allele which it carries. The phage reactions of 13 mutants were otherwise unchanged by the presence of the plasmid. The F′ derivative of the remaining mutant, SL4639, *rf.-690*, was (incompletely) sensitive to the smooth-specific phages P22, P22h, and 9NA and less sensitive than its F$^-$ parent to several rough-specific phages besides Br60 (Table 2). Correspondingly, the *sup-812*-carrying derivative of *rf.-690*, unlike its parent strain not carrying *sup-812*, gave a positive slide agglutination test for O factor 4. We conclude that *rf.-690* is partly suppressed by *sup-812*.

To determine whether the *rf.-690* site is in the *rfb* cluster or in *rfaL*, the unsuppressed mutant was crossed with SR315, a smooth donor strain which injects *his*$^+$ early (9). None of 35 recombinants selected for possession of the donor *his*$^+$ allele was smooth, as determined by tests on sensitivity to phages 9NA, 6SR, and Br60. As *rf.-690* is not closely linked to *his*, it cannot be in the *rfb* cluster and, by exclusion, is therefore probably in *rfaL*, which lies between *cysE* and *pyrE* (T. Kuo and B. A. D. Stocker, Bacteriol. Proc., p. 56, 1968; T. Kuo, Ph.D. Thesis, Stanford Univ., 1969). The *rfa* loci of some leaky *rfa* mutants which are sensitive to phage P22 are cotransducible by this phage with *cysE* and with *pyrE* (K. E. Sanderson and Y. E. Saeed, Bacteriol. Proc., p. 55, 1968). As the unsuppressed *rf.-690* strain was P22-resistant, it could not be tested in this respect. We hoped to be able to grow phage P22 on its suppressed derivative, which was P22-sensitive, but we were unable to obtain lysates of satisfactory titer. However, phage P22h, an extended host-range mutant of P22 (18), gave high-titer lysates when grown on the suppressed *rf.-690* stock—an observation which supports the theory that P22h is adsorbed by the same O-specific material as P22 but is

better adsorbed than P22 by bacteria whose lipopolysaccharide contains little O-specific material (5). Phage P22h grown on the suppressed derivative of *rf.-690* was used to evoke nonexacting transductants from two smooth recipients, SL1547 (= LT7 *cysE30 gal*) and LT2 *pyrE125*. About half of the *cys*$^+$ and about one-fourth of the *pyr*$^+$ transductants were rough, in that they were resistant to phage 9NA and sensitive to phages 6SR and Br60. As *rf.-690* is cotransducible with *cysE* and with *pyrE*, it is probably in the main *rfa* cluster, which lies between these loci and includes *rfaL* (10; T. Kuo, Ph.D. Thesis, Stanford Univ., 1969).

A *cysE*$^+$ *rfa-690* transductant derived from LT7 *cysE gal*, of phage pattern "rough-sensitive," was given the original and the suppressing forms of the F' factor by conjugation with SL4527 and SL4528, respectively, selection being made for ability to utilize galactose. The LT7 *cysE*$^+$ *rfa-690* transductant given the original F' factor was unaltered in phage sensitivity, except for resistance or partial resistance to the female-specific phages SP6 and Br60; the derivative with the suppressing form was, in addition, partly sensitive to the smooth-specific phages. Thus, *rfa-690* is partly suppressed by *sup-812* in an LT7 genetic background, as well as in its original LT2 background, and is unaffected by the original F' factor without the *sup-812* mutation.

DISCUSSION

The three ochre suppressors *sup-812*, *sup-813*, and *sup-814*, since they arose in an F'-1-*gal*$^+$ factor, must have resulted from mutation of *E. coli* genes closely linked to *gal*. Two apparently distinct ochre suppressor loci linked to *gal* have been reported in *E. coli*: *supB* (formerly su_B) and *supL* (3, 11). There is evidence that *supB* is within the chromosomal segment present in the F'-1 factor (11) and so presumably is *supL*, since it is thought to lie between *supB* and *gal* (2, 3, 15). One or other of these loci may be the same as the *E. coli* ochre suppressor locus termed *supG* (formerly *su-5*), which maps near *gal* and causes the insertion of a basic amino acid, perhaps lysine, at the position corresponding to the ochre codon (2, 4, 15). Our three suppressors may have arisen by mutation at any of these loci, but our data do not indicate which—nor do we know what amino acid(s) they determine. An amber-suppressor locus called *supE* (formerly su_{II}), causing insertion of glutamine (13), is reported to fall within the F'-1 factor and to be very closely linked to the ochre suppressor locus *supB* (11). However, our efforts to isolate an F'-*gal*$^+$ factor carrying an amber suppressor failed; the three plasmid-borne suppressors we obtained suppressed ochre as well as amber and none of 61 mutants inferred to carry amber suppressors resulted from a suppressor mutation in the F' factor. Possibly the F'-*1* factor which we used has lost some of the genes of the original factor, including the wild-type allele at the *supE*(amber suppressor) locus. However, it is not proven that *supE* specifies a transfer RNA, and if it suppresses amber mutations in *E. coli* by some other method it might not suppress them in *S. typhimurium*.

The three ochre suppressors we isolated, like others reported (14), suppressed less effectively than do some amber suppressors, as judged by the rate of growth on unsupplemented medium of suppressed derivatives of an LT2 *his*(amber) strain. Unlike some ochre suppressor mutants (2, 3), our suppressor-bearing *S. typhimurium* strains grew about as well as wild type on complete medium. This may be because they (presumably) carry on the chromosome a wild-type *S. typhimurium* allele corresponding to the mutant *E. coli* allele present in the F' factor.

The use of the F' factor bearing an ochre suppressor facilitates the testing of *S. typhimurium* mutations for suppressibility, since the suppressing F' factor is easy to introduce by conjugation, and clones which have gained the suppressor can be selected either by virtue of their galactose-positive character (if the strain to be tested is galactose-negative) or of their prototrophy (if it carries a known suppressible nutritional marker). Our detection of a suppressible *rfa* mutation illustrates the method. T. Kuo and P. Vary in this laboratory have detected many suppressible nonsmooth and nonmotile mutants of various classes by this method. In theory, the suppressing F' factor may be used in screening almost any type of mutant for suppressibility, and, because F' factors like F'-*gal* are relatively promiscuous, the system may be especially useful in strains and species other than *S. typhimurium* LT2 or *E. coli* K-12. Though we failed to isolate an F'-1-*gal*$^+$ factor carrying an amber suppressor, L. Soll (Biochemistry Department, Stanford University Medical School) has obtained a mutant F'-14-*ilv*$^+$ factor carrying an amber suppressor which can be used in *S. typhimurium* in the same way as our F'-1-*gal* ochre suppressor factor (L. Soll and P. Berg, Proc. Nat. Acad. Sci. U.S.A., *in press*). The genes carried by these suppressor-bearing plasmids, since they are of *E. coli* origin, are not exactly homologous with their chromosomal alleles in *S. typhimurium*. This nonhomology has certain advantages. Thus, loss of the suppressor allele from the F' plasmid, reported to occur frequently in *E. coli* (presumably by recombination between episome and chro-

mosome), does not occur at detectable frequency in *S. typhimurium* hosts. The suppressing F′ factors, like other F′ factors, are not completely stable in *S. typhimurium*, in that a small proportion of bacteria which have lost the whole F′-*gal*$^+$ factor are usually found. When the original *S. typhimurium* host was galactose-negative, the reappearance of the previously suppressed character in these galactose-negative segregants confirms that suppression was caused by the plasmid-borne suppressor gene rather than by, for instance, partial reversion of the chromosomal gene concerned.

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LITERATURE CITED

1. Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. Roth, and B. N. Ames. 1968. Procedure for identifying nonsense mutations. J. Bacteriol. 96:215–220.
2. Eggertsson, G. 1968. Mapping of *ochre* suppressors in *Escherichia coli*. Genet. Res. 11:15–20.
3. Eggertsson, G., and E. A. Adelberg. 1965. Map positions and specificities of suppressor mutations in *Escherichia coli* K12. Genetics 52:319–340.
4. Garen, A. 1968. Sense and nonsense in the genetic code. Science 160:149–159.
5. Gemski, P., Jr., and B. A. D. Stocker. 1967. Transduction by bacteriophage P22 in nonsmooth mutants of *Salmonella typhimurium*. J. Bacteriol. 93:1588–1597.
6. Gorini, L., and H. Kaufman. 1960. Selecting bacterial mutants by the penicillin method. Science 131:604–605.
7. Kolstad, R. A., and H. H. Prell. 1968. Amber-Mutanten des Salmonella-Bakteriophagen P22. Mol. Gen. Genet. 101:189–190.
8. Morgenroth, A., and J. P. Duguid. 1968. Demonstration of different mutational sites controlling rhamnose fermentation in FIRN and non-FIRN rha$^-$ strains of *Salmonella typhimurium*: an essay in bacterial archaeology. Genet. Res. 11:151–169.
9. Sanderson, K. E. 1965. Information transfer in *Salmonella typhimurium*. Proc. Nat. Acad. Sci. U. S. A. 53:1335–1340.
10. Sanderson, K. E. 1967. Revised linkage map of *Salmonella typhimurium*. Bacteriol. Rev. 31:354–372.
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. Smith, H. O., and M. Levine. 1967. A phage P22 gene controlling integration of prophage. Virology 31:207–216.
13. Smith, J. D., J. N. Ableson, B. F. C. Clark, H. M. Goodman, and S. Brenner. 1966. Studies on *amber* suppressor tRNA. Cold Spring Harbor Symp. Quant. Biol. 31:479–485.
14. Stretton, A. O. W., S. Kaplan, and S. Brenner. 1966. Nonsense codons. Cold Spring Harbor Symp. Quant. Biol. 31:173–179.
15. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. 31:332–353.
16. Whitfield, H. J., Jr., R. G. Martin, and B. N. Ames. 1966. Classification of aminotransferase (C gene) mutants in the histidine operon. J. Mol. Biol. 21:335–355.
17. Wilkinson, R. G., and B. A. D. Stocker. 1968. Genetics and cultural properties of mutants of *Salmonella typhimurium* lacking glucosyl or galactosyl lipopolysaccharide transferases. Nature 217:955–957.
18. Yamamoto, N., and T. F. Anderson. 1961. Genomic masking and recombination between serologically unrelated phages P22 and P221. Virology 14:430–439.
19. Yanofsky, C., and J. Ito. 1966. Nonsense codons and polarity in the tryptophan operon. J. Mol. Biol. 21:313–334.