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Genetic studies of class 2 nonsense suppressors in *Escherichia coli*

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SUMMARY

Three genetically distinct ochre suppressors have been identified in a strain of *Escherichia coli* B/r, all of which suppress a tyrosine auxotrophy and classify as class 2 by phage suppression pattern. One ochre suppressor, which was obtained by conversion from a class 2 amber suppressor, and a second ochre suppressor obtained directly from the non-suppressing parent, were found to have separate map locations, though a peculiar phenotype with regard to a leucine auxotrophy is exhibited by strains carrying either suppressor. We suggest that both suppressors correspond to separate genes for glutamine-inserting *t*RNA. A Leu$^+$ mutant of a strain carrying one of these suppressors was studied and was found to contain a further nonsense suppressor having amber-suppressing activity at a reduced level. We suggest that this suppressor might result from a mutation in another part of the translational machinery concerned with glutamine insertion. The third ochre suppressor has no effect on the leucine auxotrophy and mapping data suggest that it may be *supL*, an ochre suppressor probably inserting a different amino acid from glutamine.

1. INTRODUCTION

The three nonsense codons UAG, UAA and UGA give rise to auxotrophs because the polypeptide chain terminates at the site where the nonsense codon occurs in the messenger RNA. Revertants of such nonsense mutants may result from the appearance of external suppressors elsewhere in the DNA. Suppression of nonsense codons is considered to act at the level of translation, and mutations giving rise to transfer RNA (*t*RNA) molecules with altered anticodons have been shown to be involved in suppression. These mutant *t*RNA molecules can then recognize nonsense codons and insert amino acids at the mutant sites (for a review, see Gorini, 1970). One example of nonsense suppression resulting from an alteration in a region other than the anticodon has been demonstrated in relation to the structural gene for a tryptophan *t*RNA (Hirsch, 1971).

The conversion of amber suppressors to ochre suppressors in one mutational step provides good evidence in support of an altered anticodon model of suppression. Such evidence has been obtained for *su2* and *su3* in mutation studies by

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Ohlsson, Strigini & Beckwith (1968) and for *su1*, *su2* and *su3* by Person & Osborn (1968). Biochemical evidence for suppression by *su3* of both amber and ochre codons resulting from a single base change in the anticodon of a tyrosyl *t*RNA has since been obtained (Goodman *et al.* 1968; Altman, Brenner & Smith, 1971).

Bridges, Dennis & Munson (1970) continued the theme of interconversion of suppressors by studying the interconversion of amber and ochre suppressors in the *su2* gene, in the hope of quantitating mutation at a given single base pair.

The parent strain used in their studies was the *Escherichia coli* B/r strain WU36-10, which requires leucine and tyrosine, as a result of amber and ochre mutations respectively (Osborn & Person, 1967). From WU36-10, strains containing amber suppressors, phenotypically $Leu^{+}Tyr^{-}$, and ochre suppressors, phenotypically $Leu^{-}Tyr^{+}$, were isolated. The amber codon causing the leucine requirement in this strain appears to require stronger suppression than that mediated by the ochre suppressor which suppresses the tyrosine requirement. Strains containing the amber suppressor were classified as class 2 amber on the basis of pattern of growth of T4 nonsense mutants as described by Osborn *et al.* (1967), and ochre suppressors were similarly classified as class 2 ochre. The two types of suppressors were assumed to be allelic and, since the amber and ochre codons differ only in the third base pair, to be interconvertible in one mutational step on the anticodon model of suppression. As predicted, it was possible to isolate strains in which the amber suppressor was converted in one mutational step to an ochre suppressor. The reverse conversion, from ochre to amber suppression, could not be detected however. Obviously this observation was not in complete accord with the anticodon model of suppression.

Two problems in detecting conversions to amber were that the ochre suppressor strains derived by conversion from amber suppressors mutated to an ochre and amber suppressing phenotype at a high rate and that the leucine requirement was leaky. Ochre suppressors obtained directly from WU36-10 were found to consist of both 'unstable' ochres identical in phenotype to the amber conversions and 'stable' ochres which showed the same phage suppression activity but had normal mutation rates to Leu^{+} and were not leaky. However, no amber suppressors could be detected by conversion from either the 'stable' or the 'unstable' ochre suppressors.

In view of the different phenotypic effects of the two ochre suppressors isolated by Bridges *et al.* and the inability of these workers to detect amber suppressors by conversion from either of them, we wondered whether the two ochre suppressors were in fact identical mutations. We have used transduction to investigate this question and to study the amber-suppressing ability of a Leu^{+} derivative of the 'unstable' ochre suppressor strain. We report the results of such experiments and discuss them in the context of the anticodon model of suppression. We also suggest an explanation for the inability of Bridges *et al.* to demonstrate conversions from ochre suppressor to amber suppressor.

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2. MATERIALS AND METHODS

(i) *Bacterial strains*

Escherichia coli B/r strain WU36-10 and the isolation of its derivatives containing amber and ochre suppressors have been described previously (Bridges *et al.* 1970). For phenotypes and suppressor types see Fig. 1. Amber and ochre suppression ability is that ascribed to class 2 by Osborn *et al.* (1967). The next section analyses this classification in more detail. A Leu$^+$Tyr$^+$ spontaneous mutant of RRU6 was designated CM532 and was used throughout as a typical Leu$^+$ revertant of RRU6. Strain CM651 was isolated directly from the non-suppressing (*sup*^{wt}) parent strain WU36-10 and is an ochre suppressor strain having identical phenotypic properties to RRU6.

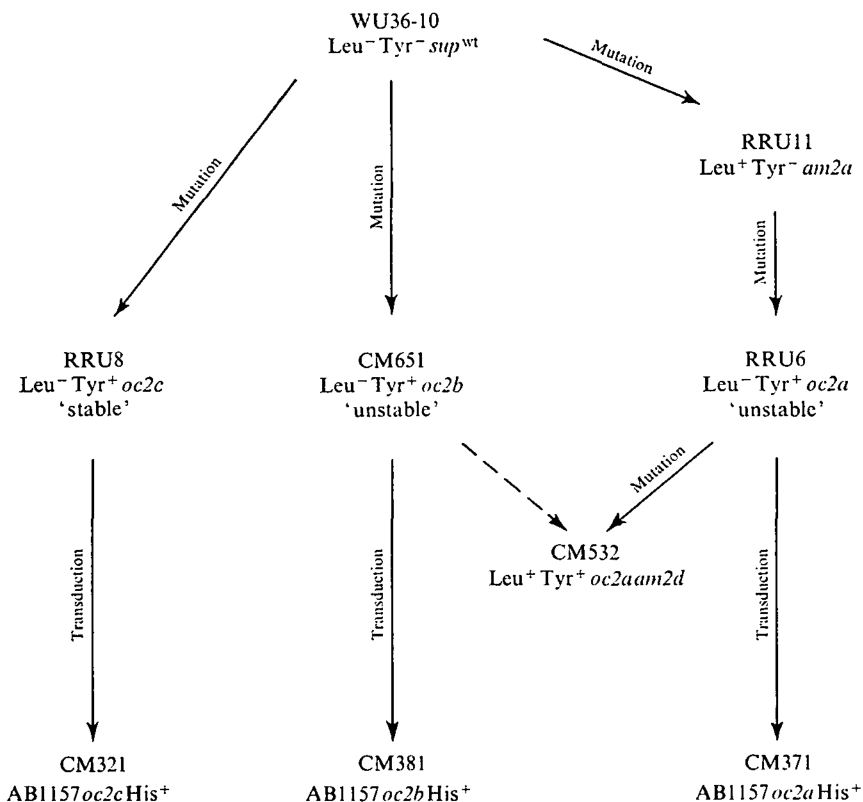

Fig. 1. Derivation and phenotypes of strains and their suppressor gene notations.

E. coli K-12 strains AB1157 *thr leu proA2 his-4 argE3 thi lac gal ara mtl xyl su2 str*^{r} and HfrH(λ)*str*^{s} were used in mapping experiments. CM321, CM371 and CM381 are derivatives of AB1157 containing the ochre suppressors of RRU8, RRU6 and CM651 respectively, and were obtained by transduction (see Results).

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(ii) *Suppressor nomenclature*

Confusion has arisen in the nomenclature of suppressor genes since different workers have used the same suppressors under a variety of names. The amber suppressor gene *suII*, which maps in the *gal* region of the *E. coli* map (Signer, Beckwith & Brenner, 1965) is assumed to be identical to the independently isolated amber suppressor gene *su2* (Garen, Garen & Wilhelm, 1965). We have used the notation *su2* to refer to this gene which inserts glutamine in response to the amber codon. Taylor (1967) adopted a further system of suppressor notation based on the Demerec convention and describes the *suII*/*su2* gene as *supE*. Osborn *et al.* (1967) classified suppressor genes on the basis of pattern of phage suppression and their class 2 amber suppressors were identified with *su2*.

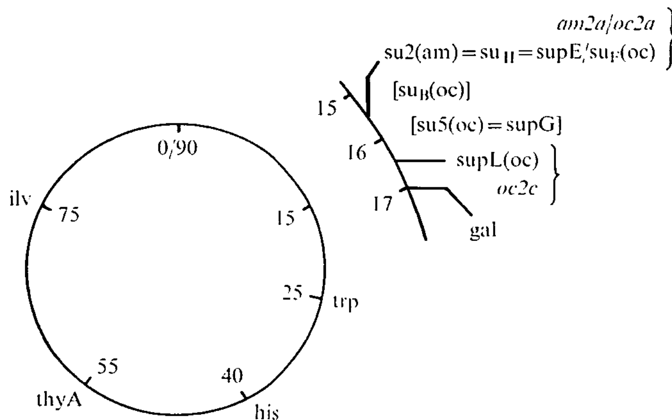

Fig. 2. Suppressor genes in the *gal* region of the *E. coli* map. Approximate map distances taken from Taylor (1970).

Ochre suppressors have been isolated directly from non-suppressing strains and also by conversion from amber suppressors in the *gal* region. Su_B was isolated by Brenner & Beckwith (1965) and maps very close to amber *su*2 (Signer *et al.* 1965) though it has been reported to be distinct from it (Ohlsson *et al.* 1968). Eggertsson & Adelberg (1965) isolated *supL*, which is an ochre suppressor mapping much closer to *gal* than su_B and *su2*. *SupL* is clearly distinguished from su_B on map position and pattern of bacterial and phage suppression (Eggertsson, 1968; Ohlsson *et al.* 1968). An ochre suppressor *su5* (which Taylor calls *sup*G) was isolated by Gallucci & Garen (1966) and was shown to be linked to *gal*. This suppressor gene was not mapped accurately but may correspond to *supL*. An ochre suppressor obtained by conversion from amber *su2* by Ohlsson *et al.* (1968) was designated su_F. Ochre suppressor strains obtained by conversion from class 2 amber suppressors by Person & Osborn (1968) were referred to as class 2 ochre suppressors by these authors. Fig. 2 summarizes the notation of allelic and non-allelic suppressor genes mapping in the *gal* region.

The present work is concerned with suppressors classifying as class 2. Since we

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show that different suppressor genes are involved in class 2 suppression, we have designated each suppressor mutation as *2a*, *b*, *c*, or *d*, prefixed by *oc* or *am*, indicating ochre or amber phage suppression ability.

Fig. 1 shows the suppressor notations for each strain and these will be referred to throughout. Non-suppressing alleles are designated sup^{wt}. The Results section shows that *am2a* is identical to amber *su2*, and *oc2a*, which was derived from *am2a* is an ochre suppressing allele of *su2* (i.e. is identical to su_F). *oc2c* and *oc2b* are ochre suppressors derived from the sup^{wt} parent strain. The suppressor *oc2c* is apparently identical to *supL*. Suppressors *oc2a*, *oc2b* and *oc2c* are all class 2 ochre suppressors on the classification of Person & Osborn (1968).

(iii) *Bacteriophage strains*

T4 nonsense mutants 55, B17, NG322, NG19 and oc427 were used. Patterns of suppression of these phages are shown in Table 1.

(iv) *Media*

Nutrient broth was Oxoid Nutrient Broth No. 2 and nutrient agar was the same medium solidified with 1·5 % Davis New Zealand Agar. L broth had the following composition: tryptone, 1 %; yeast extract, 0·5 %; NaCl, 0·5 %; glucose, 0·1 % (Lennox 1955). Tryptone agar was: Oxoid tryptone, 1 %; sodium chloride, 0·8 %, and glucose 0·1 %, solidified with 1 % agar. Sterile $CaCl_2$ was added to the agar before pouring at a concentration of M/250. Soft agar was 0·6 % Davis New Zealand Agar.

Davis & Mingioli (1950) minimal medium was used in transduction selection plates and was supplemented with glucose, 0·5 %, and citrate, 0·05 %. Additional supplements for media MLC and MTC were leucine, 20 μg/ml and tyrosine, 20 μg/ml, respectively.

Selective agar used in transducing strain AB1157 had the following supplements in addition to glucose and citrate: threonine, arginine, 50 μg/ml; proline, leucine, 25 μg/ml; thiamine, 0·25 μg/ml. In conjugation experiments, Gal^+Pro^+ selective agar contained 25 μg/ml histidine, galactose, 0·5 %, instead of glucose and 250 μg/ml streptomycin. Proline was omitted.

(v) *Plating efficiencies*

Bacteria were grown to stationary phase in L broth; 0·2 ml aliquots were mixed with 0·1 ml volumes of bacteriophage, suitably diluted and 2·5 ml of soft agar and the mixture poured on to tryptone agar plates. After overnight incubation at 37 °C phage plaques were counted and plaque forming units per ml were determined. These figures were normalized and were taken as plating efficiencies.

(vi) *Transduction*

Production of P1 transducing lysates was by the agar overlay method as described by Adams (1959), using a virulent P1 strain. P1 was cycled at least three times before being used for transduction. Transduction was performed essentially

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by the method of Lennox (1955). Recipient strains were grown overnight in L broth supplemented with M/250 $CaCl_2$. Dilutions were made into fresh medium and the cells grown to log phase. P1 transducing lysate was added at a multiplicity of infection of 1·0 and adsorption continued at 37 °C for 20 min. The mixtures were centrifuged and the supernatants, containing unadsorbed phage, discarded. The pellets were resuspended in buffer and plated on appropriate transduction selection plates.

(vii) *Spot tests for co-transduction of markers*

Transductants were picked and purified on selective agar. Purified clones were inoculated into about 0·5 ml of nutrient broth in small tubes, 50 to a rack, and the racks incubated overnight at 37 °C (Greenberg, 1967). Using capillary tubes, the cultures were streaked on to appropriate selective agar or on to nutrient agar plates spread with about 10^7 amber or ochre phages. This method of testing for unselected markers facilitated carrying out a large number of tests in as short a time as possible.

(viii) *Conjugation*

Equal volumes (2·5 ml of (*a*) an exponential culture of HfrH, (*b*) a stationary culture of CM321, CM371 or CM381, and (*c*) fresh, warmed nutrient broth were mixed gently together and incubated at 37 °C for 40 min. Mating was interrupted using a Hook and Tucker rotamixer, after which the mixture was filtered, resuspended in buffer and plated on Gal^+Pro^+ selective agar.

3. RESULTS

(i) *Suppressor activity of CM532 Leu^+Tyr^+*

Bridges, Dennis & Munson (1970) tested Leu^+ derivatives of RRU6Tyr^+Leu^- *oc2a* for amber suppressing activity with T4 nonsense mutant B17 and concluded on a qualitative basis that the revertants had gained amber suppressing activity. We examined the plating efficiencies of five T4 nonsense mutants on RRU6 and its Leu^+Tyr^+ derivative CM532. The results are shown in Table 1. It appears that whilst RRU6 and RRU8 showed no amber-suppressing activity and RRU11 showed strong amber suppression, CM532 was able to suppress amber mutations at an intermediate level. CM532 did not support the growth of NG19 suggesting the suppression was not of class 1 or class 3. Ochre suppression was exhibited by the three Tyr^+ strains RRU6, CM532 and RRU8, but not by RRU11.

(ii) *Association of amber suppressor with 'unstable' ochre suppressors in CM532*

An important question in the high frequency mutation to Leu^+ of strain RRU6 was whether the mutation to amber suppression occurred at the same site as the ochre suppressor *oc2a* in this strain. We tested this possibility by attempting to transduce the Leu^+ and Tyr^+ markers out of CM532 into WU36-10 by P1 transduction. It can be seen from Table 2A that in seven experiments using three different transducing lysates the Leu^+ marker could not be transduced into

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Table 1. *Normalized plating efficiences of five T4 nonsense mutants on strains RRU11, RRU6, CM532, RRU8 and WU36-10*

T4 nonsense mutant	Amber suppressor pattern			Bacterial strains				
	am1	am2	am3	RRU11	RRU6	CM532	RRU8	WU36-10
NG322	+	+	+	1·0	$< 5.0\times10^{-5}$	1.75×10^{-2}	5.0×10^{-5}	.
55	+	+	+	1·0	4.3×10^{-4}	8.5×10^{-3}	6.9×10^{-4}	7.8×10^{-4}
B17	+	+	Weak	1·0	$< 2.0\times10^{-5}$	9.0×10^{-3}	3.9×10^{-5}	.
NG19	+	−	+	.	$< 1.6\times10^{-5}$	1.6×10^{-5}	.	.
OC427	−	−	−	3.0×10^{-4}	1·0	9.4×10^{-1}	7.2×10^{-1}	.

Table 2A. *Transduction of leucine and tyrosine markers out of CM532*

Recipient	Expt	Colonies per plate (mean): Leu⁺ Spontaneous	Leu⁺ Transduced	Tyr⁺ Spontaneous	Tyr⁺ Transduced
WU36-10 Leu⁻Tyr⁻	1	0·7	0·3	< 1	20
	2	1·0	0·3	< 1	9
	3	4·0	1·0	2·3	155
	4	2·0	2·0	2·3	42
	5	3·7	1·3	5·3	$> 1\times10^3$
	6	0·7	2·3	< 1	860
	7	2·3	0·7	2·3	82
RRU6 Tyr⁺	1	240	$> 3\times10^3$	.	.
	2	308	$> 2.5\times10^3$	.	.
	3	123	963	.	.

Recipients were WU36-10 and RRU6. Selection for Leu⁺ transductants was on MTC agar and for Tyr⁺ transductants on MLC agar.

Table 2B. *Test for co-transduction of Leu⁺ phenotype with Tyr⁺ phenotype from CM532*

Experiment	No. Tyr⁺ transductants tested	No. Tyr⁺ transductants: Leu⁺	'Stable' Leu⁻	'Unstable' Leu⁻
1	57	1	1	55
2	55	0	1	54
5	94	1	1	92
7	87	0	3	84

Tyr⁺ transductants of WU36-10 by CM532 were picked, purified and tested for leucine phenotype.

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WU36-10 whereas the Tyr^+ marker was transduced. Table 2B shows that out of 293 Tyr^+ transductants thus obtained, 285 had the 'unstable' leucine phenotype of RRU6. The two Leu^+ and six 'stable' Leu^- isolates were probably spontaneous mutants rather than transductants. This result indicated that the mutation to amber suppression in RRU6 was due to a second mutation not at the same site as the ochre suppressor *oc2a*. We designated this amber suppressor *am2d*.

In order to establish that the Leu^+ marker could be transduced by P1, we performed the same transduction into RRU6 Leu^-Tyr^+ *oc2a*, selecting for Leu^+ transductants. Owing to the high spontaneous mutation rate to Leu^+ of RRU6, some difficulty was encountered in demonstrating Leu^+ transductants, but using a high-frequency transducing lysate there was no doubt that Leu^+ transductants of RRU6 were obtained (Table 2A). No Leu^+ transductants were obtained when RRU8 was used as the recipient (data not shown). We concluded therefore that the suppressor *am2d* of CM532 Leu^+Tyr^+ could only be expressed in the presence of the particular ochre suppressor of RRU6, *oc2a*.

Table 3. *Transduction of the Tyr^+ marker from RRU6 Tyr^+Leu^- to RRU11 Tyr^-Leu^+*

	Colonies per plate (mean)			No. Tyr^+ transductants:		
Expt	Spontaneous Tyr^+ mutants	Tyr^+ transductants	No. Tyr^+ transductants tested	Leu^+	'Stable' Leu^-	'Unstable' Leu^-
1	1·0	92	100	1	0	99
2	2·8	42	133	0	0	133

Tyr^+ transductants were selected on MLC agar and samples were picked, purified and tested for leucine auxotrophy using a spot test (see Materials and methods).

(iii) *Relationship between amber suppressor 2a of RRU11 and ochre suppressors of RRU6 and RRU8*

Since Bridges *et al.* derived RRU6 by apparent conversion from RRU11 it seemed likely that the suppressors *am2a* and *oc2a* were allelic. We tested this proposal by transducing the ochre suppressor *oc2a* (Tyr^+ phenotype) from strain RRU6 to strain RRU11 Leu^+Tyr^- *am2a*. The selective agar contained leucine, and Tyr^+ transductants were tested for leucine independence. The results of this experiment are given in Table 3. Out of 232, 231 Tyr^+ transductants tested were found to have lost their leucine independence in gaining the ochre suppressor. The one isolate which had retained its leucine independence was most probably a spontaneous Tyr^+ mutant of RRU11 not at the same site as the amber suppressor. It appeared, therefore, that the amber suppressor of RRU11 and the ochre suppressor of RRU6 were allelic since the amber suppressor *am2a* was replaced by the ochre suppressor *oc2a*. There was a possibility that this conclusion was in error since the same result would be expected if the ochre suppressor of RRU6 prevented expression of the RRU11 amber suppressor. If this were so, then the amber

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suppressor would be present but not expressed in RRU6, and would be transducible independently of the ochre suppressor, i.e. Leu^+ transductants of WU36-10 Leu^-Tyr^- would be obtained on transducing from RRU6. No Leu^+ transductants were found in experiments of this nature, although Tyr^+ was transduced (data not shown).

Thus the amber suppressor of RRU11 and the ochre suppressor of RRU6 seemed to be allelic. These observations led to the proposal that the two ochre suppressors of strains RRU6 and RRU8 *oc2a* and *oc2c* although both classified as class 2 (Osborn & Person, 1967; Bridges *et al.* 1970) might in fact be two different suppressors. In order to test the non-allelism of the RRU11 amber suppressor with the RRU8 ochre suppressor, we transduced the Leu^+ marker from RRU11 Leu^+Tyr^- *am2a* to RRU8 Leu^-Tyr^+ *oc2c* and tested the Leu^+ transductants for tyrosine independence. Table 4 shows that the amber suppressor *am2a* was transduced into RRU8 without concommitant loss of the ochre suppressor *oc2c*, thus these two suppressors appear not to be allelic.

Table 4. *Transduction of the Leu^+ marker from RRU11 Leu^+Tyr^- to RRU8 Leu^-Tyr^+*

Expt	Colonies per plate (mean): Spontaneous Leu^+ mutants	Colonies per plate (mean): Leu^+ transductants	No. Leu^+ transductants tested	No. Leu^+ transductants: Tyr^+	No. Leu^+ transductants: Tyr^-
1	1·0	25	25	25	0

Leu^+ transductants were selected on MTC agar and samples were picked, purified and tested for tyrosine auxotrophy using a spot test.

(iv) *Mapping of ochre suppressors*

The *E. coli* K-12 strain AB1157 contains an amber suppressor believed to be *su2* (Signer *et al.* 1965; George & Devoret, 1971) which classifies as class 2 amber in the classification of Osborn *et al.* (1967). AB1157 has a histidine requirement which can be suppressed by at least four ochre suppressors (Eggertsson, 1968).

We transduced the ochre suppressors from RRU6 and RRU8 into AB1157 selecting for His^+ and tested the His^+ transductants for ochre and amber suppression using T4 nonsense mutants B17 and *oc427*. Table 5 shows that the RRU6 ochre suppressor *oc2a* substituted for the amber *su2* of AB1157, confirming the classification of this ochre suppressor as allelic with *su2*. As predicted the RRU8 ochre suppressor *oc2c* did not substitute for the amber suppressor. In these crosses the His^+ transductants not receiving the ochre suppressor were due to transduction of the structural his^+ gene.

Having thus obtained His^+ derivatives of AB1157 containing the RRU8 ochre suppressor *oc2c* (strain CM321) and the RRU6 ochre suppressor *oc2a* (CM371) it was possible to map these suppressors by mating with the K-12 donor strain HfrH.

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Gal^+Pro^+ recombinants were selected, since the region of the chromosome containing amber *su2* and other suppressors is bounded by these markers. Colonies having the phenotype Pro^+Gal^+ were purified and tested for unselected markers, the sup^{wt} alleles of the amber and ochre suppressors. The results are presented in Tables 6 and 7. The likeliest order of markers in CM321 is *proA am2a oc2c gal* since only this order gives the $oc2c\,am^{wt}$ class as very rare. Thus *oc2c* would appear to coincide with *supL* as indicated in Fig. 2.

Table 5. *Transduction of ochre suppressors of RRU6* (oc2a) *and RRU8* (oc2c) *into AB1157* su2

Donor	No. His^+ transductants tested	His^+ transductants: oc	am	No.
RRU6	50	+	−	20
		−	+	30
RRU8	94	+	+	44
		−	+	50

His^+ transductants were purified and tested for amber and ochre suppression with T4 mutants B17 and oc427.
+, Phage growth.

Table 6. *Gal^+Pro^+ recombinants of CM321: test for unselected markers am* $2a^{wt}$ *and oc* $2c^{wt}$

No. Gal^+Pro^+ recombinants tested	Classes of recombinants: $oc^{wt}am^{wt}His^-$	$oc2c\,am2\,His^+$	$oc^{wt}am2\,His^-$	$oc2c\,am^{wt}His^+$
297	277	6	12	2

Recombinants were tested for loss of amber and ochre suppressors using T4 nonsense mutants B17 and oc427.

Table 7. *Gal^+Pro^+ recombinants of CM371: test for unselected marker oc* $2a^{wt}$

No. Gal^+Pro^+ recombinants tested	Classes of recombinants: $oc^{wt}His^-$	$oc2a\,His^+$
49	48	1

Recombinants were tested for loss of ochre suppressor using T4 nonsense mutant oc427.

(v) *Ochre suppressor with associated instability isolated directly from* sup^{wt} *strain*

In their search for ochre to amber interconversions, Bridges *et al.* (1970) sought ochre suppressors directly from the sup^{wt} parent strain WU36-10. They reported that about half of these were of the RRU8 type, i.e. with 'stable' leucine phenotype, the remainder showing the 'unstable' phenotype of RRU6. We isolated one of the latter variety, designated CM651 and transduced the ochre suppressor of

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this strain into AB1157. Table 8 shows the results of this transduction. Of 12 His$^+$ transductants of AB1157 in which the ochre suppressor overcame the histidine requirement, all had retained their original amber *su2*. Thus the ochre suppressor obtained directly from the *sup^{wt}* gene of WU36-10, whilst exhibiting the same 'unstable' leucine phenotype as the RRU6 ochre suppressor *oc2a* was due to a mutation at a separate site from *oc2a* and we designated it *oc2b*.

Table 8. *Transduction of ochre suppressor of CM651* (oc2b) *into AB1157*

No. His$^+$ transductants tested	His$^+$ transductants: ochre	amber	No.
47	+	+	12
	+	−	0
	−	+	35

His$^+$ transductants were purified and tested for amber and ochre suppression with T4 mutants oc427 and B17.
+, Phage growth.

Table 9. *Gal$^+$Pro$^+$ recombinants of CM381: test for unselected markers am* 2^{wt} *and oc* 2b^{wt}

No. Gal$^+$Pro$^+$ recombinants tested	Classes of recombinants: *oc^{wt}am^{wt}*His$^-$	*oc^{wt}am^{wt}*His$^+$	*oc2b am^{wt}*His$^+$
44	19	2	23

Recombinants were tested for loss of amber and ochre suppressors using T4 nonsense mutants B17 and oc427.

One of the AB1157 *am2oc2b* derivatives thus isolated was designated CM381. This strain was used as recipient in the same type of conjugation experiment as was performed with CM321 and CM371, selecting for Gal$^+$Pro$^+$ recombinants. The recombinants were purified and analysed for loss of amber and ochre suppressors and for loss of His$^+$ phenotype. If the ochre suppressor of CM381 mapped distal to *gal* from the origin, in contrast to the ochre suppressors of CM321 and CM371,we predicted a class of recombinants having received the *sup^{wt}* gene, i.e. lacking ochre suppression ability but also being His$^+$ due to the structural *his$^+$* gene from the donor. This class was not found in the previous conjugation experiments since the suppressor genes entered much earlier than *his$^+$*. Results of recombinant analysis are given in Table 9. The ochre suppressor was clearly not linked to *gal*, as demonstrated by the majority class of *oc2b am^{wt}*His$^+$ recombinants. Two recombinants receiving *oc^{wt}* were His$^+$ due to the structural *his$^+$* gene. The most likely map position for the ochre suppressor *oc2b* is in the segment between *gal* and *his*.

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4. DISCUSSION

It has been shown in previous studies (Osborn & Person, 1967; Person & Osborn, 1968; Bridges *et al.* 1970) that the tyrosine requirement of strain WU36-10 can be suppressed by an ochre suppressor designated class 2, assumed to be allelic with amber *su2*. We have now demonstrated that three genetically distinct ochre suppressors, all classifying as class 2 on phage testing (Bridges *et al.* 1970), are involved in suppression of the tyrosine auxotrophy, only one of which is allelic with amber *su2*.

The amber suppressor of strain RRU11 *am2a* we have demonstrated to be allelic with the 'unstable' ochre suppressor of strain RRU6 *oc2a*. Thus the amber to ochre interconversion studied by Osborn & Person (1967) and by Bridges *et al.* (1970) can be assumed to be a true interconversion, probably in the DNA coding for the anticodon of a glutamine inserting *t*RNA (Kaplan, Stretton & Brenner, 1965; Weigert, Lanka & Garen, 1965; Wilhelm, 1966; Gesteland, Salser & Bolle, 1967). On the other hand, the ochre suppressor of RRU8 *oc2c* which classifies on phage suppressor pattern as identical to *oc2a* of RRU6, is not allelic with amber *su2*. In the RRU8 ochre suppressor strain the leucine auxotrophy is not leaky, which also suggests that a different suppressor gene might be involved. Our mapping data by conjugation implicates *supL* (Eggertsson & Adelberg, 1965; Eggertsson, 1968) as an identification for *oc2c* (see Fig. 2). Although it has been reported that *supL* does not suppress the T4 ochre mutant oc427 (Ohlsson *et al.* 1968) we have found that a strain containing *supL* (AB2300, kindly supplied by Dr Barbara Bachmann, *E. coli* Genetic Stock Center) does suppress oc427 under our conditions (unpublished data). The mapping data are not consistent with the identification of the RRU8 ochre suppressor as su_B, an ochre suppressor very close to or allelic with amber *suII* (Brenner & Beckwith, 1965; Signer *et al.* 1965)

Not all ochre suppressors have corresponding amber suppressing alleles and no amber suppressor is known which maps at the same genetic site as *supL* (Taylor, 1970). There is presumably a restriction on amber suppressor formation at this locus; it is not known which amino acid is inserted by *supL* or whether a *t*RNA gene is involved, only that on genetic grounds the pathway of suppression by *supL* appears to be different from that by *su2*, su_B and su_F (Ohlsson *et al.* 1968; Eggertsson, 1968). An ochre suppressor conversion to an amber suppressor in one mutational step at this site might therefore be unlikely. This is the most probable explanation for the absence of ochre to amber conversions reported by Bridges *et al.* (1970) in strain RRU8.

The ochre suppressor *oc2a* found in strain RRU6 exhibits a peculiar phenotype with respect to the leucine requirement, which is leaky and has an elevated mutation rate to Leu^+. This 'unstable' type is clearly very much associated with the interconverted ochre suppressor since when this suppressor is transduced into strain RRU11 replacing the amber suppressor, the transductants are Tyr^+Leu^- but all exhibit the 'unstable' leucine phenotype (Table 3).

The amber suppression in the Leu^+Tyr^+ derivative of RRU6, CM532, was

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studied. On a plating efficiency test of T4 nonsense mutants, CM532 was found to exhibit increased suppression of amber mutants, at a level intermediate between no suppression and efficient suppression such as by RRU11 *am2a*. The amber suppressing component of CM532 *am2d* is, however, found to be a mutation at a site separate from *oc2a* of RRU6. Furthermore, *am2d* seems to be expressed only in a strain already containing *oc2a* since the Leu$^+$ phenotype can be successfully transduced from CM532 into RRU6, the strain from which it was derived, but not into WU36-10. Thus, although *am2d* of CM532 functions only in the presence of *oc2a* it is a genuine genetic alteration which maps separately and can be transduced back into its parent strain RRU6. Although we have designated it as an amber suppressor on account of the additional amber-suppressing activity in strain CM532, it could conceivably be an ochre suppressor mediating increased efficiency of suppression. No increase in ochre-suppressing activity has been detected in CM532, however.

The same properties of 'unstable' leucine phenotype shown by RRU6 are shown by about half the ochre suppressor strains obtained directly from the parent strain WU36-10 (the remainder correspond to the 'stable' ochre suppressor RRU8). The ochre suppressor *oc2b* of one such isolate, CM651, was shown not to be allelic with the amber *su2* of AB1157 since ochre-suppressing transductants of AB1157 by CM651 were found to have retained the amber suppressor, in contrast to transductants by RRU6. In other words, the two mutations giving rise to ochre suppressor genes directly from sup^{wt} and by conversion from an amber *su2* gene, whilst giving identical peculiar phenotypes with respect to the leucine mutation, are not at the same site. The amber *su2* gene has been shown to insert glutamine in response to the amber codon, the suppressing component being a *t*RNA, probably a glutamine *t*RNA (Kaplan *et al.* 1965; Weigert *et al.* 1965; Wilhelm, 1966; Gesteland *et al.* 1967). There are in *E. coli* two species of glutamine-inserting *t*RNA's each of which has an anticodon specific for only one of the two possible codons for glutamine, CAG and CAA (Folk & Yaniv, 1972). This being so, it is not possible, on the anticodon model of suppression, for both an amber and an ochre suppressing *t*RNA to arise in a single step from either of the two glutamine *t*RNA genes. The amber suppressor *am2a* of RRU11 identical to *su2*, has most likely arisen as a result of a mutation in the anticodon of the *t*RNA specific for CAG enabling it to recognize UAG instead of, or in addition to, CAG. The ochre-suppressing allele of *su2*, *oc2a*, would be a further mutation in the anticodon, altering the codon recognition from UAG to UAA. *oc2b* was isolated directly from sup^{wt} and has the same phenotypic effect on the leucine auxotrophy as *oc2a*. Since *oc2b* is a different gene from *oc2a* it might then be a mutation at the anticodon of the other glutamine *t*RNA gene specific for the codon CAA, this anticodon now recognizing UAA. Both these anticodon mutations would involve glutamine *t*RNA genes, which are structurally very similar apart from the difference in anticodon specificities. We therefore suggest that the associated leucine 'instability' is brought about by some component common to glutamine *t*RNA genes which have mutated to ochre suppression. Candidates for this function might

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be amino acyl activating enzymes or ribosomal components, one possibility being that mutations permitting ochre suppression by the *t*RNA cause conformational changes which exert effects on other biological functions of the molecule.

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