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Mapping of *ochre* suppressors in *Escherichia coli*

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1. INTRODUCTION

Suppressors of the *ilv-188* mutation in *E. coli* were studied by Eggertsson & Adelberg (1965) and were found to map at four different loci: *supL*, *supM*, *supN* and *supO*. Suppressors representing these four loci were shown to suppress the lac_2 mutation, which is known to be an *ochre* mutation (Brenner & Beckwith, 1965). These suppressors are therefore referred to as *ochre* suppressors.

In this paper further work bearing on the characterization of suppressors of *ilv-188* is described.

2. MATERIALS AND METHODS

Bacterial strains. The following derivatives of *E. coli* K 12 were used (only relevant genetic markers are listed):

AB 2300 Hfr *ilv-188 supL2 gal*$^+$ *bio*$^+$ (Eggertsson & Adelberg, 1965).
AB 2550 F$^-$ *ilv-188 metE46* lac_2 (*lacZ13*) *try-3 his-4* (Eggertsson & Adelberg, 1965).
AB 2567 Hfr *ilv-188 supN23 aroC*$^+$ *purC*$^+$. A derivative of strain K 10 obtained from Dr A. Garen.
AB 2568 F$^-$ *argF*$^+$ *supM*$^+$ *purD26 his-4*. derived by transducing the *argF*$^+$ allele into strain AT 1380 with phage P 1.
AB 2587 F$^-$ *argF1 supM20 purD*$^+$ (Eggertsson & Adelberg, 1965).
AB 2594 F$^-$ *ilv-188 supL*$^+$ *supN*$^+$ *gal-2 argF*$^+$ *aroC*$^+$ *try*$^+$. Derived from AB 2291 (Eggertsson & Adelberg, 1965) by introducing the *try*$^+$ allele by transduction with phage P 1.
AB 2595 Hfr *ilv-188 supL*$^+$ *supN*$^+$ *aroC8 purC1 try-24*. Derived from strain AB 2270 (Eggertsson & Adelberg, 1965) by inducing the *aroC8* and *try-24* mutations with ethylmethanesulfonate (EMS).
AT 1380 F$^-$ *argF1 supM*$^+$ *purD26 his-4*. Obtained from Dr A. Taylor.
GE 100 F$^-$ *ilv-188 supL*$^+$ *gal-30 bio-5*. Derived from strain A 437 obtained from Dr S. E. Luria.
CA 168 Hfr su_B^+ lac_2 (*lacZ13*). Obtained from Dr J. Beckwith.
CA 169 Hfr su_C^+ lac_2 (*lacZ13*). Obtained from Dr J. Beckwith.

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Genetic symbols refer to loci concerned with the biosynthesis of isoleucine and valine (*ilv*), methionine (*met*), arginine (*arg*), purines (*pur*), biotin (*bio*), tryptophan (*try*), histidine (*his*) aromatic amino acids (*aro*), and with the utilization of lactose (*lac*) and galactose (*gal*); *sup* or *su* = suppressor locus; λ = bacteriophage λ.

The map positions of the genetic markers used are shown in Fig. 1. The rules of nomenclature suggested by Demerec, Adelberg, Clark & Hartman (1966) are followed. Note that the '*sup*' symbol followed by a '+' sign (for instance *supM*$^+$) refers to a wild-type allele (without suppressor-function) of a suppressor locus while *sup* followed by an allele number refers to a mutant allele (with suppressor-function) of a suppressor locus.

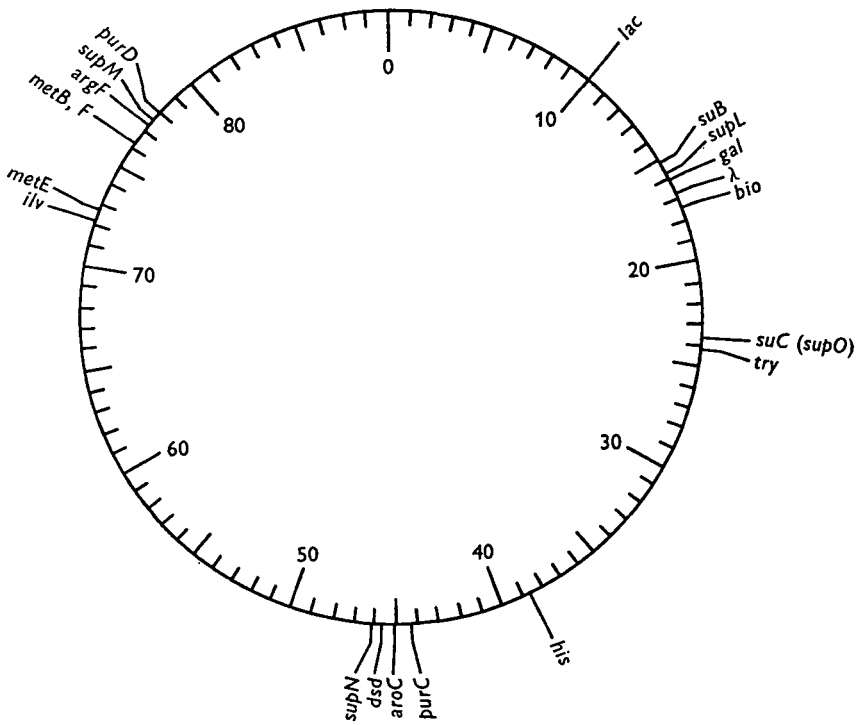

Fig. 1. Genetic map of *Escherichia coli* showing the position of *ochre* suppressor loci. The basic design of the map is that of Taylor & Thoman (1964).

Media and culture methods: as described by Adelberg & Burns (1960).

Transduction by phage P1kc (referred to as P1); essentially as described by Lennox (1955).

3. RESULTS AND DISCUSSION

(i) *Mapping of the* supM *locus*

The *supM* locus was previously shown to be co-transducible by P1 with the *argF* locus and with the *met-27* marker which represents either *metB* or *metF* (Eggertsson & Adelberg, 1965). The order of these loci was established as *met–*

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argF–supM. In order to determine more exactly the map position of *supM* the experiment described in Table 1 was carried out. The results show conclusively that *supM* is located between the *argF* and *purD* loci which were co-transduced at a frequency of about 25%. The co-transducibility of *purD* and *supM* was confirmed in a transduction experiment in which strain AT1380 (*supM*$^+$ *purD26 his-4*) was used as recipient and strain AB2587 (*supM20*, *purD*$^+$) as donor: 56% of 200 *purD*$^+$ transductants scored carried the *supM20* allele.

Table 1. *Linkage of* argF, supM *and* purD *determined by transduction with P1*

Unselected markers scored	Transductants	Co-transduction with *purD*$^+$ (%)
argF1	74	24·6
*supM20**	192	64·0
argF$^+$ *supM20*	119	—
argF$^+$ *supM*$^+$	107	—
argF1 supM20	73	—
argF1 supM$^+$	1	—

Recipient AB2568 (*argF*$^+$ *supM*$^+$ *purD26 his-4*); donor AB2587 (*argF1 supM20 purD*$^+$). Selection was made for *purD*$^+$ and 300 transductants scored for the unselected *argF* and *supM* markers.

* Scored for ability to grow without histidine supplementation (the *his-4* mutation is suppressed by *supM20*).

Table 2. *Linkage of* supN, aroC *and* purD *determined by transduction with P1*

Selected marker	Number of transductants scored	Transductants carrying unselected marker (%)		
		supN23	*aroC*$^+$	*purC*$^+$
supN23 (Isoval$^+$)	200	—	2·0	1·5
aroC$^+$	500	2·4	—	56

Recipient: AB2595 (*supN*$^+$ *aroC8 purC1 ilv-188*). Donor: AB2567 (*supN23*, *aroC*$^+$, *purC*$^+$, *ilv-188*).

(ii) *Mapping of the* supN *locus*

The *supN* locus was previously shown to be located near *purC*, and was shown to be transferred earlier than that locus by an Hfr strain which transfers its chromosome with the marker order *purC–his–try* (Eggertsson & Adelberg, 1965). The data presented in Table 2 show that *supN* is co-transducible at a frequency of about 2% with both *aroC* and *purC*, but clearly not located between these two loci. The order of *aroC* and *purC* in relation to the *his* locus has been determined by McFall (1967) as *aroC–purC–his*. Taking into consideration the results of the transfer experiments referred to above, the order *supN–aroC–purC–his* is established. Preliminary transduction experiments indicate that the locus for D-serine deaminase (the *dsd* locus) is located between *supN* and *aroC* (McFall, 1967; G. Eggertsson, unpublished).

(iii) *Mapping of the* supL *locus*

The *supL* locus was shown by Eggertsson & Adelberg (1965) to be co-transducible with *gal* by phages P1 and lambda at frequencies of approximately 65% and 2% respectively. Insertion of phage lambda occurs between the *gal* and *bio* loci (Rothman, 1965). Experiments carried out to determine the position of *supL* in relation to *gal* and *bio* are described in Table 3. The data strongly indicate that *supL* is located distal to *gal* from the *bio* locus. This location of *supL* was expected on the basis of the previous observation that *supL* was not transduced independently of *gal* by lambda; i.e. transductants which received *supL* invariably also received the *gal* marker of the donor (Eggertsson & Adelberg, 1965).

Table 3. *Linkage of* supL, gal *and* bio *determined by transduction with P1*

Selected marker	Total no. of transductants scored	Unselected markers scored	Transductants	Co-transduction with selected marker (%)
A *supL2* (Isoval+)	67	*gal*+	37	55·2
		bio+	11	16·5
		gal+ *bio*+	10	—
		gal+ *bio*−	27	—
		gal− *bio*+	1	—
		gal− *bio*−	29	—
B *gal*+	150	*supL2*	97	64·7
		bio+	41	27·3
		supL2 bio+	27	—
		supL2 bio−	70	—
		supL+ *bio*−	39	—
		supL+ *bio*+	14	—

Recipient GE100 (*supL*+ *gal-30 bio-5 ilv-188*); donor AB2300 (*supL2 gal*+ *bio*+ *liv-188*). A and B each represents a separate experiment.

(iv) *Further mapping of suppressors of* ilv-188

In the study by Eggertsson & Adelberg (1965) suppressors which were mapped at the *supL* or *supN* loci could not be distinguished on the basis of their phenotypic effects. They were referred to collectively as suppressors of type 1, whereas suppressors at the *supO* and *supM* loci were referred to as suppressors of type 2 and type 3, respectively. In the present study the question whether suppressors of type 1 may occur at loci other than *supL* or *supN* was examined further. Twenty-four Isoval+ revertants having the phenotypic characteristics associated with suppressors of type 1 were induced by EMS in strain AB2594 (which carries *ilv-188*) and tested for the presence of suppressors of *ilv-188* co-transducible with the *aroC* or *gal* loci. In these tests the Isoval+ revertants were used as P1 donors and strain AB2595 as recipient. Nineteen of the revertants carried suppressors which were co-transducible with *aroC* at frequencies comparable to that found for *supN23* (ranging from 1·7%–6·1% for 180 transductants scored). The re-

maining five revertants contained suppressors which were co-transducible with *gal* at frequencies similar to that found previously for *supL* (ranging from 56%–64% for 100 transductants scored). These twenty-four revertants are therefore thought to be due to suppressors either at *supN* (19) or *supL* (5).

(v) *Tests of suppression by* su_B^+ *and* su_C^+ *of* ilv-188

The *ochre* suppressors su_B^+, su_C^+, su_D^+ and su_E^+, which all suppress the lac_2 mutation, were described by Brenner & Beckwith (1965). Two of these suppressors, su_B^+ and su_C^+, have been tested for ability to suppress *ilv-188*. P1 lysates of strains CA 168 and CA 169, which carry su_B^+ and su_C^+ respectively, were used to transduce these suppressors into strain AB 2550, which carries lac_2 and *ilv-188*. Selection was made for the Lac$^+$ phenotype and transductants scored for suppression of *ilv-188*. Su_B^+ did not suppress *ilv-188* and therefore differs in specificity from the *supL*, *supM*, *supN* and *supO* suppressors. It also differs from these suppressors with respect to its map location, being co-transducible with *gal* by P1 at a frequency of about 1% (Signer, Beckwith & Brenner, 1965). On the other hand, su_C^+ suppressed *ilv-188*, giving the same phenotypic characteristics as the *supO* suppressors. Similar co-transduction frequencies with *try* have been found for both *supO* and su_C (Eggertsson & Adelberg, 1965; Signer *et al.* 1965), suggesting that both designations may refer to the same locus. The relationship of su_D and su_E to *supM* and *supN* is unknown. Two additional *ochre* suppressors, *Su-4*$^+$ and *Su-5*$^+$ were described and mapped by Gallucci & Garen (1965) and further characterization of *Su-4*$^+$ was given by Brenner, Kaplan & Stretton (1966). The *Su-4* locus was co-transduced with *try* at a frequency of about 50% and may be identical to su_C and/or *supO*. The *Su-5* locus was co-transduced with *gal* at a frequency of about 10% and may therefore possibly be identified with either su_B or *supL*. Thus, the number of *ochre* suppressor loci in *E. coli* is at least five as was concluded by Signer *et al.* (1965).

SUMMARY

Genetic mapping of suppressors of the *ilv-188* mutation is described. Suppressors of this mutation have been mapped at four *ochre* suppressor loci: *supL*, *supM*, *supN* and *supO*. The su_B^+ *ochre* suppressor described by Brenner & Beckwith is shown not to suppress *ilv-188* whereas the su_C^+ suppressor described by the same authors suppresses *ilv-188* and may represent the same locus as *supO* suppressors. The mapping by P1 transduction of the *supL*, *supM* and *supN* loci is described.

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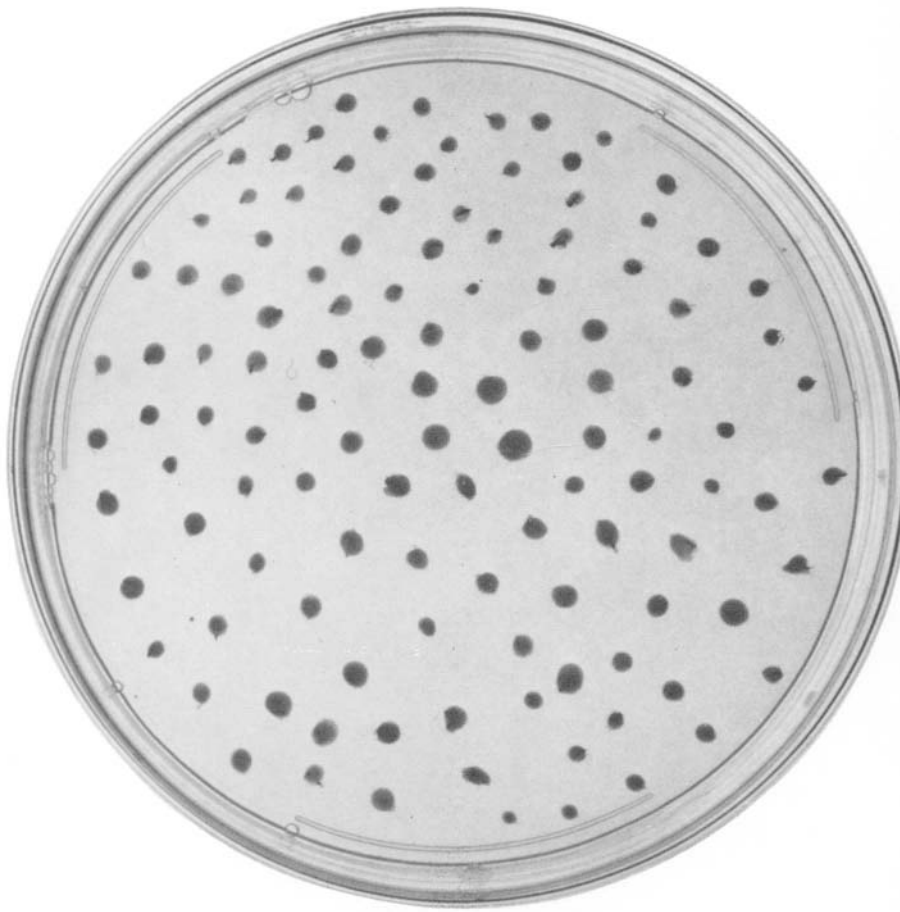

Colonies of *Eudorina elegans* (strain 62f) after 1 week of growth on minimal agar.

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