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New Suppressor in *Escherichia coli*[1]

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During the genetic mapping of a mutation in the *pheS* gene which confers temperature sensitivity on a strain of *Escherichia coli* K-12, an extragenic suppressor was discovered which restores ability to grow at the restrictive temperature. The suppressor, which has been named *supQ*, is cotransduced by bacteriophage P1 with the *purE* marker. *SupQ* does not suppress a number of amber or ochre mutations. *SupQ*$^-$ is carried by the prototrophic Hfr Hayes strain AB259, and the presence of the *supQ*$^-$ allele impairs the growth of this strain at 42 C.

Approximately 20 loci on the *Escherichia coli* K-12 chromosome have been implicated in the suppression of a variety of mutations (1, 8, 10). Although, in many cases, the ability of a strain to suppress has either been shown or inferred to be due to the recognition of the "nonsense" amber or ochre codons by an altered transfer ribonucleic acid (tRNA), there are also instances in which the suppressors do not correct nonsense mutations. In two such cases, suppression has been inferred to be due to altered ribosomes (1, 8, 7) or to a changed tRNA which responds to a missense codon (3). At least two further suppressors have been reported which have not been shown to suppress amber or ochre mutations, and in which the cellular change resulting in suppression has not been identified (5, 6). This paper describes the recognition of a new suppressor carried by an Hfr Hayes strain, the genetic mapping of the suppressor gene, and some observations on the effect of the *sup*$^-$ allele on cell growth.

MATERIALS AND METHODS

Those used are described in the accompanying paper (Russell and Pittard, J. Bacteriol., *submitted for publication*). All organisms used were strains of *E. coli* K-12 and are described in Table 1.

Figure 1 shows the derivation of the major strains to which we refer, and Figure 2 shows the map positions of the mutations and to the origins of transfer of the Hfr strains used.

RESULTS

The selection and study of a number of temperature-sensitive mutants are described elsewhere (Russell and Pittard, *submitted for publication*). Mutant JP1112 was isolated from KA56 after treatment with nitrosoguanidine. JP1112, although isolated as a temperature-sensitive mutant from KA56, lost its maleness at some stage during mutant isolation and purification. To map its *ts* mutation, therefore, a spontaneous streptomycin-resistant mutant, JP1420, was prepared and used as recipient in interrupted conjugation experiments, with Hfr strains of various origins as donors. Figures 3 and 4 show the results of crosses between JP1420 and two Hfr strains which transfer chromosome with opposite orientation, AB259 and JP243 (Fig. 2). All interrupted mating experiments were performed at 32 C, at which temperature the rate of chromosome transfer is approximately half of that at 37 C. Concurrently with the results of the above ge-

TABLE 1. *Organisms used*[a]

Strain	Sex	Origin of transfer (min)	Genotype
AB259	Hfr H	88	*thi*$^-$, *supQ*$^-$
AB1515	F$^-$		*thi*$^-$, *leu*$^-$, *proC*$^-$*14*, *purE*$^-$ *trp*$^-$, *lac*$^-$, *str*^{r}
AB2332	Hfr C	13	*thi*$^-$, *met*$^-$
AB3311	Hfr R1	74	*thi*$^-$, *met*$^-$
CA292	Hfr C	13	*lac* amber, *trp* amber, *supF*$^-$
CAJ (64)	F$^-$		Contains UGA suppressor
E508	F$^-$		*supE*$^-$
JP243	Hfr O-311	38	*proA-2*
JP259	Hfr H	88	*thi*$^-$
JP342	Hfr H	88	*thi*$^-$
JP1112	F$^-$		*thi*$^-$, *galE-PL5*, *pheS-353*
JP1420	F$^-$		*thi*$^-$, *galE-PL5*, *pheS-353*, *str*^{r}
JP1440	F$^-$		*thi*$^-$, *purE*$^-$, *pheS-353*, *his-4*, *argE-3*, *gal*$^-$, *lac*$^-$, *str*^{r}
KA56	Hfr H	88	*thi*$^-$, *galE-PL5*
W112	F$^-$		*pro*$^-$, *supB*$^-$, *supQ*$^-$

[a] Gene symbols are those used by Taylor (10).

[1] These results are taken from a Thesis submitted by R.R.B.R. in partial fulfillment of the requirements for a Ph.D. Degree.

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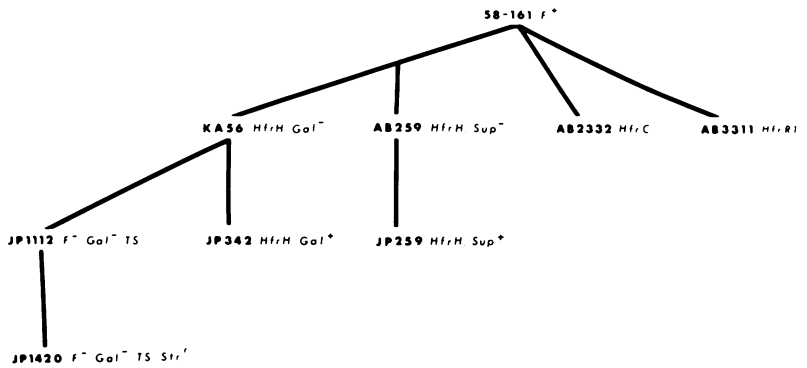

FIG. 1. *Genealogy of some of the strains referred to in this paper, showing the characters of particular interest.*

netic experiments, biochemical tests revealed that the phenylalanyl-tRNA synthetase of JP1112 was temperature sensitive. The gene determining this enzyme, *pheS*, had recently been reported by Böck and Neidhardt (2) to be at minute 33 on the genetic map, a position consistent with that indicated by the JP1420 × JP243 cross. In confirmation of these results, the *ts* mutation of JP1112 was shown to be cotransducible with *aroD* at a frequency of 43%.

These findings showed that *pheS-353*, the mutation responsible for the temperature sensitivity of JP1112 (and its derivatives), mapped closely to *aroD*, some 16 min distant from *gal* on the map. The cross JP1420 × AB259, however, indicated that some other gene on the donor chromosome close to *gal* was capable of yielding TS^+ recombinants. This suggested that AB259 carried a suppressor of *pheS-353;* it also indicated that TS^+ recombinants formed early in the JP1420 × AB259 cross would contain the suppressor, while retaining the *pheS-353* mutation. This was tested by growing phage P1 on one such early TS^+ recombinant and using it to transduce Aro^+ into an $aroD^-$ strain. Temperature-sensitive Aro^+ transductants were obtained, confirming the presence of the *pheS* mutation in the TS^+ recombinant.

The suppressor in AB259 has been named *supQ*, i.e., AB259 carrying an active suppressor is $supQ^-$, whereas JP1420 is $supQ^+$. Since AB259 and KA56 (from which JP1112 was derived) are both Hfr Hayes strains of common parentage, it was of interest to determine whether KA56 was also $supQ^-$. Figure 3 shows that in a JP1420 × JP342 (a spontaneous Gal^+ derivative of KA56) cross no TS^+ recombinants are formed until the predicted time-of-entry of the $pheS^+$ allele, thus KA56 must be $supQ^+$.

SupQ is located on the counterclockwise side of the *gal* locus and can be placed approximately 2 min from the *gal* locus on the conventional (37 C) map (Fig. 3). *SupQ* is not cotransducible

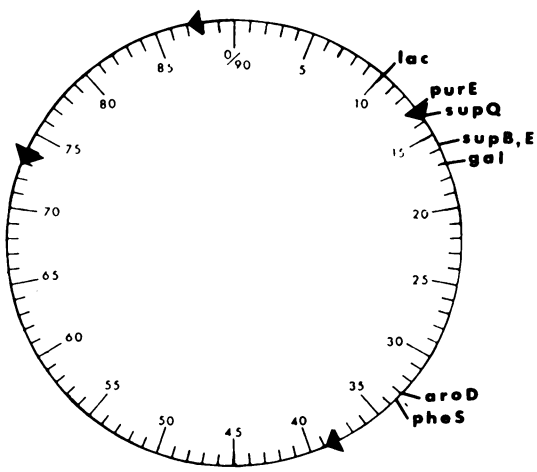

FIG. 2. *Genetic linkage map of E. coli, based on that of Taylor (10), showing positions of relevant markers and origins of transfer of Hfr strains. The origins of transfer of the Hfr strains used are listed in Table 1.*

with *gal*, but *supQ* and *purE* are cotransduced at a frequency of 45%, since 52 of 116 transductants were TS^+ when P1 grown on AB259 was used to introduce $purE^+$ into strain JP1440. A cross between JP1440 and AB259 showed further that the order of genes was *lac purE supQ* (Table 2).

Recent reports (4, 10) put *purE* and *gal* 4 min apart. However, at the time of the work described here, available data had them separated by only 2 min. This latter arrangement suggested that *supQ* lay very close to the known suppressors of nonsense mutations, *supE* and *supB*. Since a nonsense mutation resulting in temperature sensitivity would be both novel and unexpected, experiments were prepared to determine whether (i) *supQ* could suppress nonsense mutations and (ii) *pheS-353* was suppressible by nonsense suppressors.

(i) Strain JP1440, containing the pheS-353

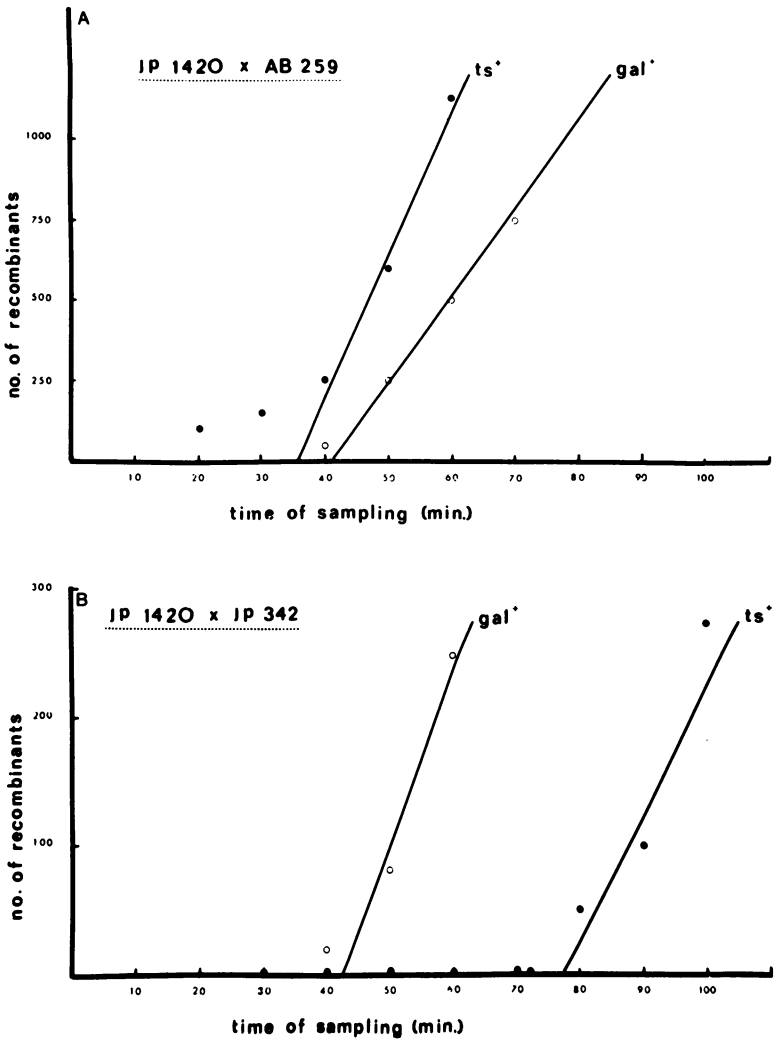

FIG. 3. *Kinetics of zygote formation at 32 C in the crosses between JP1420 and the two HfrH strains, AB259 which is supQ⁻ (A) and JP342 which is supQ⁺ (B). Recombinants were selected for their ability to grow on galactose as sole carbon source (Gal⁺) or at 42 C (TS⁺).*

mutation and also the ochre mutation *his-4*, was prepared by a series of conjugation and transduction experiments (*data not given*). Transductants of JP1440 which were *purE⁺ supQ⁻* (and thus TS⁺) retained their His⁻ phenotype. Similarly, when *purE⁺* was transduced from AB259 into *purE⁻* strains which carried an amber lac mutation (from CA292) or the ochre *ilv-188* mutation, no suppression of these was found.

(ii) Preparations of P1 grown on the four suppressor-carrying strains listed in Table 3 were used to transduce *purE⁺* into JP1440, and transductants were inspected for their ability to grow at 42 C. Only AB259 and W112 were found to contain a suppressor of *pheS*-353, and, in both cases, the suppressor is 40 to 45% cotransducible with *purE* (Table 3). W112, therefore, appears to carry *supQ⁻* (or another suppressor capable of suppressing *pheS-353*), but this is distinct from the other suppressor it carries, *supB*. This is shown by the fact that no *purE⁺* transductants from a W112 donor had *his-4* suppressed. *SupB* cannot, therefore, be cotransduced with *purE* although it is cotransduced, at a low frequency, with *gal* (reference 9; Table 3) which again distinguishes it from *supQ*.

The strain AB259 shows poor growth at 42 C on minimal medium. The locus responsible for this partial temperature sensitivity has been shown to be in the *gal* region of the chromosome, and a transductant of AB259 which has the ability to grow well at 42 C has been pre-

pared (E. M. Walker, *unpublished data*). Although AB259 grows at 42 C at only one-quarter of its rate at 32 C, the AB259 TS$^+$ transductant (named JP259) grows equally well at the two temperatures.

An examination of strain JP259 has shown that in gaining an ability to grow well at 42 C, this strain has lost its ability to suppress *pheS-353*. This suggests that it might be the *supQ*$^-$ allele (or some closely linked gene) which alters the growth rate. Further evidence that it is in fact *supQ* which is involved comes from the finding that three of eight transductants of AB1515 which received *purE*$^+$ from AB259 also exhibit a decreased growth rate at 42 C.

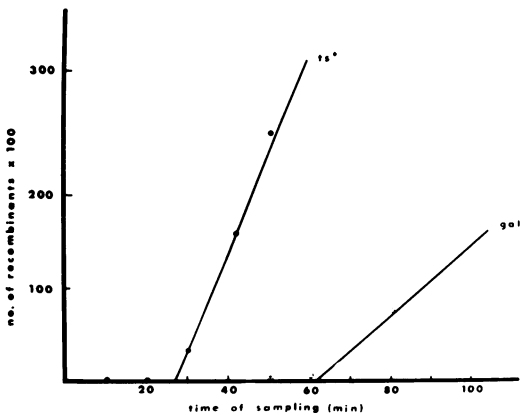

FIG. 4. *Kinetics of zygote formation at 32 C in the cross between JP1420 and the Hfr strain JP243, which has its origin of transfer at minute 38.*

TABLE 2. *Results of an uninterrupted mating between JP1440 and the HfrH strain AB259, in which Lac$^+$ recombinants were selected*

Two possible gene orders

	1	2	3		1	2	3
	lac	purE	supQ		lac	supQ	purE
(A)←				(B)←			
	+	+	−		+	−	+
	−	−	+		−	+	−

Recombinant classes obtained				Regions containing crossovers in	
lac	*purE*	*supQ*	No. in class	Model A	Model B
+	−	+	98	1	1
+	+	+	37	2	1, 2, 3
+	+	−	40−	3	3
+	−	−	0	1, 2, 3	2

DISCUSSION

There is no evidence as to the nature of the function coded for by *supQ*. Without testing a wider range of nonsense suppressors, it is not possible to decide whether the *pheS-353* mutation is a member of one of the nonsense classes. However, the results presented here show that if the mutant codon is recognized by amber or ochre suppressors, neither glutamine (inserted by *supE*) nor the amino acid inserted by *supB* is an acceptable amino acid at the site in the phenylalanyl-tRNA synthetase molecule altered by the *pheS-353* mutation. Conversely, the ability of *supQ* to suppress a variety of known nonsense mutations would need to be checked before the conclusion that *supQ* did not suppress any amber or ochre mutations could be reached. The *supQ*$^-$ allele of AB259 does not suppress two other *pheS* mutations (*pheS-354* and *pheS-360; submitted for publication*), thus it does not appear to be gene-specific. Thus far, the *supQ*$^-$ allele has not been found to suppress any mutation other than *pheS-353*.

Suppressor mutations have previously been observed by several workers to affect aspects of the messenger RNA translation and protein-synthesizing system (recently discussed by Eggertsson, reference 5). *SupQ* may well exert its effect at the level of translation, but it is also possible that its suppressor activity is due to the alteration of some cellular structure or component so as to result in the stabilization of the mutant enzyme in *pheS-353* cells. This alteration, although it restores phenylalanyl-tRNA synthetase activity, would, however, have a deleterious effect on the cell as a whole at elevated temperatures.

The origin of the *supQ*$^-$ mutation in AB259 is

TABLE 3. *Test for suppression of pheS-353, his-4, or lac amber mutations carried by purE$^-$ or gal$^-$ recipient strains*

Strain	Known suppressor	Codons suppressed	Per cent of Pur$^+$ transductants which were			Per cent of Gal$^+$ transductants which were		
			TS$^+$	His$^+$	Lac$^+$	TS$^+$	His$^+$	Lac$^+$
AB259	*supQ*	?	45	0	0	0	0	0
E508	*supE*	amber (UAG)	0		0	0	0	1
W112	*supB*	ochre (UAG, UAA)	40	0	0	0	2	3
CAJ (64)		opel (UGA)	0			0	0	0

not clear. It has been shown how another Hfr Hayes strain, KA56, is *supQ*$^+$. Two other Hfr strains with a common ancestry to AB259, namely AB2332 and AB3311, also are *supQ*$^+$. All of these Hfr strains which do not have suppressor activity can grow well at 42 C, further illustrating the deleterious effects on growth attributed to the *supQ*$^-$ allele.

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