SUPPRESSORS CAUSING TEMPERATURE SENSITIVITY OF GROWTH IN *ESCHERICHIA COLI*

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IN a study of suppressors of *ilv* mutations in *E. coli* a suppressor locus, *supH*, was shown to be co-transducible by phage P1 with a *his* locus (EGGERTSSON and ADELBERG 1965). In this paper suppressors which map at the *supH* locus are shown to have temperature-dependent inhibitory effects on cell growth and bacteriophage multiplication. Data bearing on the specificity of these suppressors are presented.

MATERIALS AND METHODS

Media: The minimal medium used was a half-strength preparation of medium 56 described by MONOD, COHEN-BAZIRE and COHN (1951), supplemented with glucose or lactose (2mg/ml) and as necessary with amino acids (100–200 μg/ml) and adenine (20 μg/ml). For agar plates this medium was supplemented with 1.5% Bacto agar. This medium was used for selection of revertants and transductants and for testing nutritional requirements.

L broth (LURIA and BURROWS 1957) was used for routine preparation of bacterial cultures. For agar plates this medium was supplemented with 2% Bacto agar.

Tryptone broth (10g Bacto tryptone, 5g NaCl per liter) was supplemented with 0.7% Bacto agar for use in phage assays.

Bacteria: The bacterial strains used are listed in Table 1. They are all derivatives of *E. coli* K-12.

Bacteriophages: 1) Phage P1kc (LENNOX 1955) was used for transduction of bacterial markers and is referred to as P1. 2) Phage T4—The *amber* mutants of T4 used were among those described by EPSTEIN *et al.* (1963). 3) Wild-type phage λ.

Induction of mutants: Auxotrophic mutations and Lac$^-$ mutations (causing inability to utilize lactose) were induced by ethylmethane-sulfonate (EMS) and isolated by penicillin selection. Diethylsulfate (DES) was used for induction of Ilv$^+$ revertants (of strain AB2096) as described by EGGERTSSON and ADELBERG (1955). Concomitant reversion to Ilv$^+$ and Lac$^+$ in Lac$^-$ mutants of strain GE196 was tested for by plating 0.1 ml of overnight cultures (in L broth) on plates containing minimal lactose medium without isoleucine and valine supplementation and adding 0.02 ml of EMS to the center of the plates. The plates were incubated at 30°C for 4–5 days.

P1 transduction: Methods used in preparing P1 stocks and in transduction experiments with P1 were essentially those described by LENNOX (1955).

Burst sizes: T4: cells grown to 4×10^8/ml in L broth at 30°C were infected with about 5×10^6 T4 particles per ml. Before infection the sample to be infected (1.8 ml) was equilibrated at the appropriate temperature for 1 min. Five min after the addition of phage the infected culture was diluted 10^{-4} into prewarmed broth. At 60 min lysis was induced with chloroform.

λ: cells grown to about 10^8/ml in L broth at 30°C were irradiated with UV for induction of λ. Samples of the irradiated culture were then incubated in the dark at 37°C, 42°C and 43°C and lysed with chloroform after 100 min.

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TABLE 1

Bacterial strains used

Strain No.	Mating type	Suppressor present*	Other genetic markers*		Source or reference
AB1931	Hfr	u.a.	*metE46*	$(\lambda)^-$	EGGERTSSON and ADELBERG (1965)
AB1953	Hfr	u.a.	*ilv-88*	$(\lambda)^-$	same reference
AB2273	Hfr	u.a., *supH11*	*ilv-145*	$(\lambda)^-$	same reference
AB2285	Hfr	u.a., *supH12*	*ilv-88*	$(\lambda)^-$	same reference
AB2096†	Hfr	u.a.	*ilv-145*	$(\lambda)^-$	same reference
GE143†	Hfr	u.a., *supH25*	*ilv-145*	$(\lambda)^-$	From AB2096 by DES
AB2277	F^-	u.a.	*metE46, ilv-145, his-4, try-3*		EGGERTSSON and ADELBERG (1965)
GE196	Hfr	none (Pm^-)	*ilv-145*	$(\lambda)^+$	From strain K10 of A. GAREN‡
GE199	Hfr	*supH25*	*ilv-145*	$(\lambda)^+$	From GE196 by P1-transduction of *supH25*
S26R1E	Hfr	*Su-1*$^+$		$(\lambda)^+$	GAREN, GAREN and WILHELM (1966)
S26R1D	Hfr	*Su-2*$^+$		$(\lambda)^+$	same reference
H12R8a	Hfr	*Su-3*$^+$		$(\lambda)^+$	same reference

* Only those genetic markers are given which are relevant for the experiments described in this paper. Genetic symbols refer to loci concerned with the biosynthesis of isoleucine and valine (*ilv*), methionine (*met*), histidine (*his*), tryptophan (*try*); *su* or *sup* = suppressor locus; $(\lambda)^-$ and $(\lambda)^+$ = absence and presence of bacteriophage λ, respectively; u.a. = unidentified *amber* suppressor. Pm^- = suppressors not known to be present.

† λ-lysogenic derivatives of these strains were also used.

‡ Derived from strain K10 by the following steps: 1) induction of an *ilv* mutation; 2) introduction of *metE46* by co-transduction with *ilv*$^+$; 3) introduction of *ilv-145* by co-transduction with *metE*$^+$.

Tests of temperature sensitivity of cell growth: Cells were routinely tested for temperature sensitivity by plating 0.1 ml of a 10^{-2} dilution of an overnight broth culture on L agar plates which were subsequently incubated at 43°C for 24 hrs. Transductants to be tested for temperature sensitivity were transferred to plates of the selective medium which, after overnight incubation at 30°C, were replicated onto L agar plates for incubation at 43°C overnight.

Optical density was measured at 550 mμ in the Coleman Universal Spectrophotometer.

Pulse-labelling with C^{14} labelled precursors: A log phase culture grown in tryptone broth at 32°C to about 2×10^7 cells/ml was transferred at time 0 to a water bath shaker equilibrated at 43°C. Samples (2 ml) were taken at different times after the temperature shift and incubated for 2 min with 0.05 μC of the labelled precursor: C^{14} leucine (specific activity 250 μC/μmole) C^{14} thymidine (30 μC/μmole) and C^{14} uracil (30 μC/μmole). (The C^{14} labelled precursors were purchased from the New England Nuclear Corp.) After 2 min, 2 ml of cold 10% TCA was added. The samples were filtered on millipore filters, dried and counted in the SELO low background counter.

Tests of suppression of T4 amber mutants by supH25: The ability of these mutants to form plaques on a strain containing the suppressor was used as a criterion for suppression.

Nomenclature: The rules of nomenclature suggested by DEMEREC, ADELBERG, CLARK and HARTMAN (1966) are followed. Note that the symbols Ilv+ and Ilv− (ability and inability to grow without isoleucine and valine supplementation, respectively) refer to phenotypes only.

RESULTS

Mapping of suppressors of ilv-145: In a previous study (EGGERTSSON and ADELBERG 1965), two suppressors of the *ilv-145* mutation, *supH11* and *supH12*, were

mapped at a locus, *supH*, which was shown to be co-transducible with a *his* marker at a frequency of 4–6%. Subsequently, the *supH* locus was shown by HOWARD-FLANDERS, BOYCE and THERIOT (1966) to be located between the loci *uvrC* and *his*; co-transduction frequencies of 15.7% and 17% were found for the *his-supH* and *supH-uvrC* pairs, respectively.

The present investigation of suppressors of *ilv-145* was prompted by the observation that certain isoleucine-valine independent (Ilv$^+$) revertants of strain AB2096 showed unusual temperature sensitivity of growth. Among spontaneous and DES-induced Ilv$^+$ revertants of this strain obtained at 30°C, two phenotypic classes were distinguished: 1) revertants which grew slowly at 30–37°C both on the selective medium and on minimal or nutrient media containing isoleucine and valine; 2) revertants which under the same conditions grew at rates comparable to that of wild type. 60 independently isolated revertants of class 1 and 10 of class 2 were tested for ability to grow at 43°C on L agar. All of the former failed to form colonies at this temperature whereas all of the latter grew normally.

To test for the presence of suppressors at the *supH* locus in Ilv$^+$ revertants of class 1 seven such revertants were used as donors in transduction experiments with AB2277 (*ilv-145*, *his-4*) as recipient. Selection was made for the Ilv$^+$ phenotype and transductants scored for this *his*$^+$ marker of the donor. Each of these revertants was found to contain a suppressor of the *ilv-145* mutation that was co-transducible with *his* at a frequency of about 10% (Table 2). In each of these experiments, all the Ilv$^+$ transductants scored were temperature sensitive like the donors, confirming for these particular revertants that the suppression of *ilv-145* and the temperature sensitivity trait are both determined by the same mutation.

Strains AB2273 and AB2285 which carry the *supH11* and *supH12* mutations, respectively, are both capable of growing on L agar at 43°C. However, it was found that both the *supH11* and *supH12* mutations of these strains caused temperature sensitivity when transduced into strain AB2277 (Table 2). Strains AB2273 and AB2285 were also used as donors in transduction experiments with

TABLE 2

Mapping of suppressors of ilv-145 *by co-transduction with* his

Donor	Suppressor-allele	Number of Ilv$^+$ transductants scored	Percent *his*$^+$	Percent temperature sensitive
AB2096 Rev. 7	*supH25*	100	10	100
AB2096 Rev. 51	*supH26*	100	12	100
AB2096 Rev. 53	*supH27*	100	12	100
AB2096 Rev. 54	*supH28*	100	10	100
AB2096 Rev. 56	*supH29*	100	8	100
AB2096 Rev. 57	*supH30*	100	10	100
AB2096 Rev. 60	*supH31*	100	9	100
AB2273	*supH11*	200	11	100
AB2285	*supH12*	200	9	100

Strain AB2277 (*ilv-145 his-4*) was used as recipient. Strain AB2096 carries *ilv-145* and *his*. All the Ilv$^+$ revertants of AB2096 were obtained after treatment with DES.

AB2096 as recipient. In each case, selection was made for Ilv$^+$ (*supH11* or *supH12*) transductants, 100 of which were tested for temperature sensitivity; all were found to be temperature sensitive. The ability of strains AB2273 and AB2285 to grow at 43°C is, therefore, probably due to the presence of mutations outside the *supH* locus (termed "secondary reversions") which counteract the temperature sensitivity effect of the *supH11* and *supH12* mutations. Selection for such mutations may have occurred during the isolation of these strains which was carried out at 37°C. (See a later section: *secondary revertants of strains carrying* supH25) The co-transduction frequencies with *his* found for *supH11* and *supH12* in the experiments described above are closely similar to the co-transduction frequencies found for the other suppressors of *ilv-145* which were mapped (Table 2). Along with the identical phenotypic effect (temperature sensitivity) of these suppressors, the mapping data strongly suggest that they are all mutations of the same locus, the *supH* locus.

Most of the additional experiments to be reported in this paper were carried out using strains carrying the allele *supH25* originally present in AB2096 Rev7 (Table 2). To avoid the possible effect of additional mutations that might have been induced by DES during selection for this revertant, the *supH25* allele was transduced into AB2096, thus strain GE143 was obtained. The *supH25* allele was also transduced into the Pm$^-$ strain GE196; thus strain GE199 was obtained (Table 1).

Transduction tests were carried out with two of the Ilv$^+$ revertants of class 2 (revertants L2 and L8) to determine if the Ilv$^+$ phenotype of these revertants is due to external suppressors of the *ilv-145* allele. These tests were based on the finding that the *ilv* and *metE* loci are co-transducible at a frequency of about 50% (EGGERTSSON and ADELBERG 1965). Using these Ilv$^+$ revertants as P1 donors and strain AB1931 (*ilv*$^+$, *metE46*) as recipient, *metE*$^+$ transductants were obtained and scored for the unselected Ilv phenotype. In each case, no isoleucine-valine dependent (Ilv$^-$) transductants were found among 100 transductants scored. It can, therefore, be concluded that the Ilv$^+$ phenotype of these revertants is not due to external suppressors but rather caused by mutations at the *ilv* locus, possibly true back mutations. When in a control experiment, a donor lysate of strain GE143 (which carries *supH25*) was used, 55 of 100 *metE*$^+$ transductants scored were Ilv$^-$.

Effect of supH25 *in the absence of* ilv-145: A test was carried out to determine whether the temperature sensitivity effect associated with *supH25* is dependent on the presence of the *ilv-145* mutation. The *supH25* allele was transduced into strain AB2277 which carries *ilv-145* and *metE46*. A strain thus obtained (which was temperature sensitive) was used as recipient in a transduction test with an *ilv*$^+$ *metE*$^+$ donor. Selection was made for *metE*$^+$ transductants at 30°C on medium containing isoleucine and valine, and transductants were scored for temperature sensitivity. Of 100 transductants scored all were found to be temperature sensitive. As about 50% of these are expected to have received the *ilv*$^+$ allele of the donor by co-transduction with *metE*$^+$, it is concluded that temperature sensitivity is caused by *supH25* independently of the presence of *ilv-145*.

Temperature sensitivity of cell growth and macromolecular synthesis: When cultures of strains carrying *supH25* were plated on L agar and incubated at 43°C for 48 hours, only approximately $5 \times 10^{-3} - 10^{-4}$ of the cells plated grew to form colonies. On further incubation of the plates at lower temperature (30°C) for 24 hours no further increase in the number of colonies was observed, showing that at this temperature the growth-inhibitory effect of the suppressor-mutation is lethal. Closely comparable results were obtained with all the 60 slow-growing revertants of strain AB2096 which were tested for temperature sensitivity (see above). The nature of the colonies obtained at 43°C is discussed in a later section of this paper.

Figure 1 shows the effect of incubation in tryptone broth at 43°C (after a shift from 32°C) on growth and survival of strains GE143 and GE199 both of

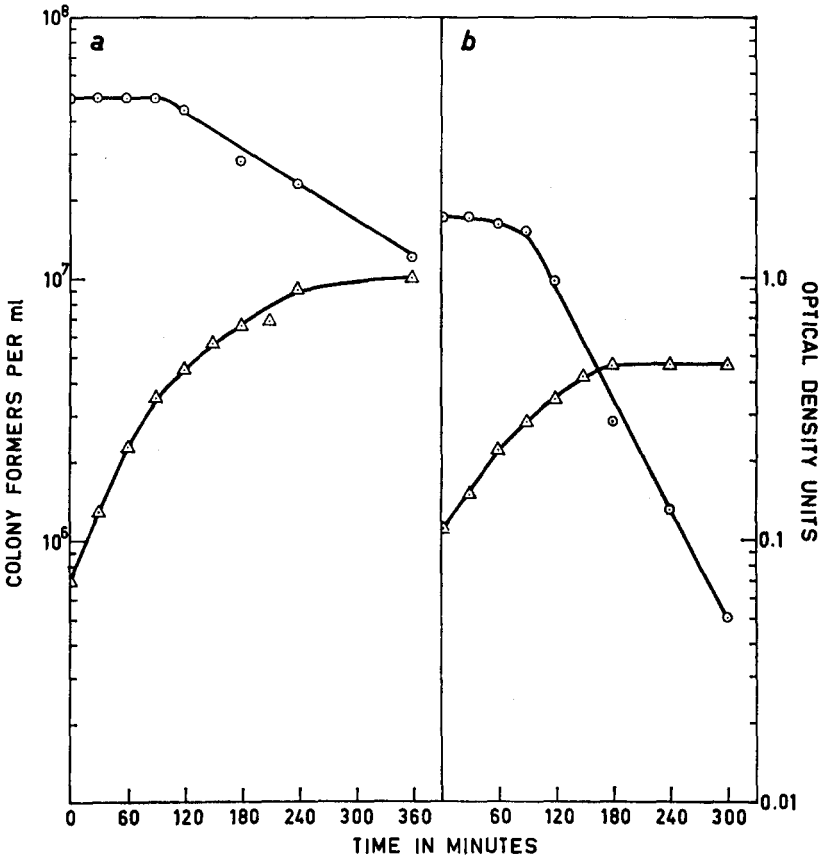

FIGURE 1.—Effect of incubation at 43°C on growth and survival of strains carrying *supH25*. Cultures of GE199 (a) and GE143 (b) grown to log phase in tryptone broth at 32°C were incubated with aeration at 43°C at time 0. Corrections are made for dilutions that were made into equal volumes of prewarmed broth at intervals when O.D. had reached approximately double the original value. △–△–△–△ optical density units. O–O–O–O viable counts on L agar plates incubated at 30°C.

which carry *supH25*. In the case of strain GE143, the optical density (O.D.) of the cultures increased about fivefold before a level was reached after approximately 180 min of incubation at 43°C. In the case of strain GE199, the O.D. continued to increase at least for 240 min after incubation at 43°C, having increased about fifteenfold during this period. In neither strain was there any increase in viable counts at 43°C. Microscopic observations of the cultures showed that a large proportion of the cells grew to form long filaments. In both strains a decrease in the number of viable cells was noted after 120 min of incubation at 43°C, while the O.D. of the cultures was still increasing. However, the rate of killing was slower in strain GE199 which showed the greater increase in O.D. On plates (L agar or tryptone agar) survival of these two strains at 43°C was comparable.

In the experiment of Figure 1b the synthesis of DNA, RNA and protein was followed by pulse-labelling with C^{14} labelled precursors (Figure 2). It is seen that a gradual decrease in the rate of synthesis of each of these components took place after the shift from 32 to 43°C. In a control experiment with strain AB2096,

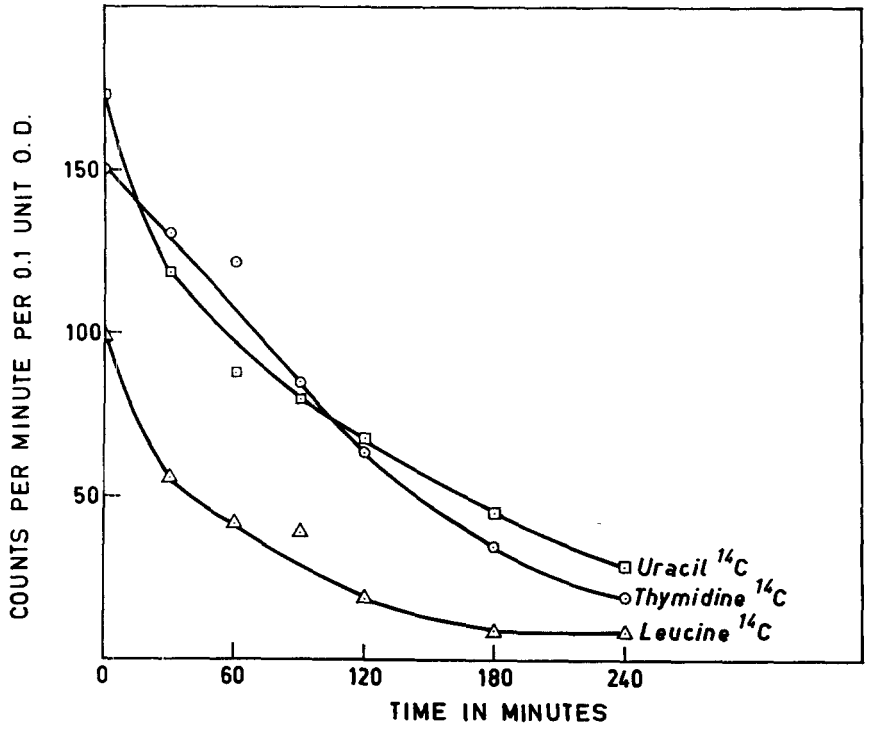

FIGURE 2.—Synthesis of macromolecules in strain GE143 at 43°C. Synthesis of DNA, RNA and protein in strain GE143 was followed by pulse labelling with C^{14} labelled thymidine, uracil and leucine, respectively, after a shift from 32° to 43°C of the culture represented in Figure 1b. Experimental procedures are described in MATERIALS AND METHODS. O–O–O C^{14} thymidine; □–□–□ C^{14} uracil; △–△–△ C^{14} leucine.

TABLE 3

Effect of supH25 *on growth*

Bacterial strain	*supH25*	Doubling time in L broth (in minutes) 30°	37°	43°
AB2096	absent	35	. .	20
GE143	present	47	. .	no multiplication
GE196	absent	44	23	22
GE199	present	57	34	no multiplication

the rates of synthesis of DNA, RNA and protein did not decrease significantly during 240 min of incubation at 43°C.

In strain GE199 growth inhibition and survival on L agar plates at 42°C were found to be approximately the same as at 43°C. The inhibition of growth gradually decreased as the temperature of incubation was lowered from 41°C to 39°C and at 38°C viability of plated cells was the same as at 30°C.

The data presented in Table 3 show that in strains carrying *supH25* growth rates were reduced also at 37°C and 30°C.

Temperature sensitivity of bacteriophage growth: The results presented in Table 4 show the effect of temperature on the multiplication of bacteriophages T4 and λ in strains carrying *supH25*. The average burst size of phage T4 obtained at 43°C in the strains carrying *supH25* was less than 5% of that obtained at the same temperature in the control strains carrying the wild-type *supH* allele or at 37°C in the strains carrying *supH25*. At 42°C the yield of T4 was intermediate between the yields at 37°C and at 43°C. The production of λ after UV-irradiation of strain GE143 $(\lambda)^+$ was strongly inhibited at 43°C. Also in the case of λ, the inhibitory effect of *supH25* was considerably weaker at 42°C than at 43°C.

Figure 3 shows the effect of temperature shifts from 37°C to 43°C on phage production by T4 infected cells of the *supH25* strain GE199. It is seen that in strain GE199 the temperature shift caused a considerable reduction in phage yield even if performed at a late stage in phage development (10–15 min after phage infection). With strain GE196 the inhibitory effect of early temperature

TABLE 4

Effect of temperature on burst size of phages T4 and λ *in strains carrying* supH25

Bacterial strain	*supH25*	T4 37°	T4 42°	T4 43°	λ 37°	λ 42°	λ 43°
AB2096	absent	145.	180.	132.	. . .	. . .	. . .
GE143	present	84.	9.2	2.3	. . .	. . .	. . .
GE196	absent	140.	. . .	95.	. . .	. . .	. . .
GE199	present	90.	20.	1.1	. . .	. . .	. . .
AB2096$(\lambda)^+$	absent	. . .	. . .	. . .	150.	. . .	15.
GE143$(\lambda)^+$	present	. . .	. . .	. . .	141.	3.4	0.004

Each datum represents the average of at least two experimental results.

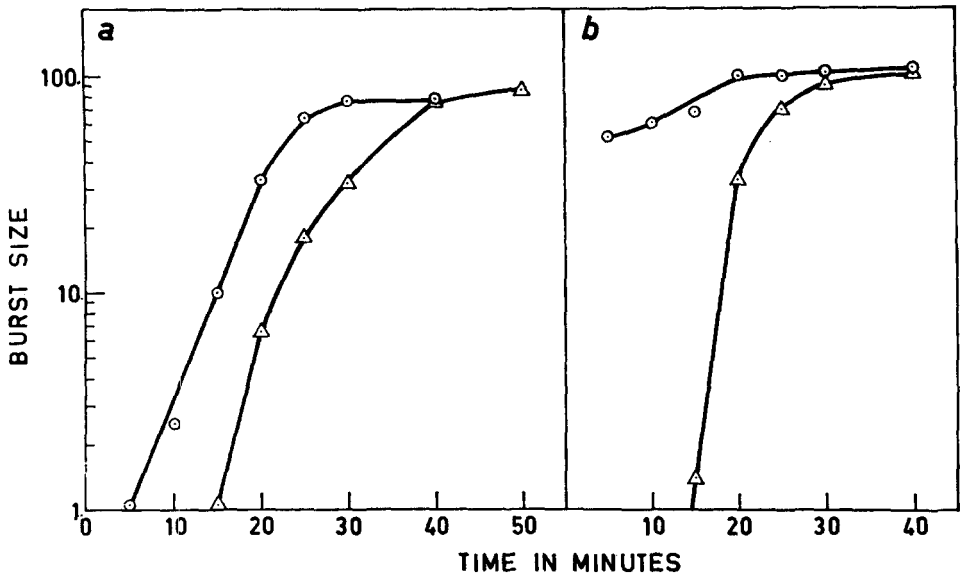

FIGURE 3.—Effect of *supH25* on burst size of T4 after shift from 37°C to 43°C. Log phase cultures of 2–4 × 10^8 cells/ml (in L broth) were infected with T4 at a multiplicity of approximately 0.01 at 37°C. At 5 min the infected culture was diluted 10^{-4} in L broth. Samples (1 ml) were taken at intervals and incubated at 43°C; at the same time samples were taken, lysed with chloroform and assayed for phage. The samples incubated at 43°C and the culture at 37°C were lysed at 60 min; a) strain GE199; b) strain GE196. O–O–O–O burst size at 60 min after infection in cells having undergone temperature shift at time indicated by point. △–△–△ burst size at time of temperature shift.

shifts was much less marked. It is noted that at 37°C phage development is somewhat slower in GE199 than in GE196.

Shifting of T4 infected cells of GE199 from 37°C to 43°C for "pulses" of 10 min at the latter temperature during early or late stages of phage development did not significantly alter the final yield of phage (Table 5).

Secondary revertants of strains carrying supH25: Among three hundred col-

TABLE 5

Effect of 10 min pulses of incubation at 43°C on burst size of T4 in strains GE199 and GE196

Time of pulse (min after infection)	Burst size in	
	GE199	GE196
0–10	55	65
5–15	60	70
10–20	61	67
15–25	51	65
20–30	54	72
no temp. shift	52	68

Except for the incubation of 1 ml samples at 43°C for ten minutes as indicated, these experiments were carried out as described for determinations of burst sizes in MATERIALS AND METHODS.

onies of strain GE143 obtained on L agar at 43°C and referred to as "secondary revertants" two main phenotypes were observed: A) Ilv⁻ with growth rates comparable to AB2096; B) Ilv⁺, more slow-growing than AB2096. On further transfer both types were capable of growing on L agar at 43°C. Revertants of type B (100 tested) were Ilv⁺ at 37°C, but at 43°C they needed supplementation of isoleucine and valine for growth. Thus, suppression of *ilv-145* is not effective at 43°C in these revertants.

Two revertants of each type, A and B, were tested for the presence of the *supH25* allele. P1 lysates were prepared on these revertants and used in transduction tests with AB2096 as recipient. Selection was made for Ilv⁺ transductants at 30°C. No such transductants were obtained when the revertants of type A were used as donors indicating that these revertants are due to mutations within the *supH* locus, possibly true back mutations. In tests with the two revertants of type B as donors, Ilv⁺ transductants were obtained and were found to be temperature sensitive like GE143; in each case all of 100 transductants tested were temperature sensitive. The ability of these revertants of type B to grow at 43°C is, therefore, due to mutations located outside the *supH* locus. Some characteristics of secondary revertants of GE143 are summarized in Table 6. It is recalled that the ability of strains AB2273 and AB2285 to grow at 43°C had previously been shown to be due to mutations outside the *supH* locus (Table 2).

It is concluded that colony formation at 43°C in strains carrying *supH25* is dependent on the occurrence of mutations ("secondary reversions") which counteract or reverse the lethal action of *supH25*; these mutations can be located either within the *supH* locus or outside it.

Specificity of supH *suppressors:* Among 12 *ilv*⁺ mutations previously tested for suppression by the *supH11* mutation, only two (*ilv-145* and *ilv-88*) were found to be suppressed (EGGERTSSON and ADELBERG 1964). It is not known whether *ilv-145* and *ilv-88* represent different mutational sites. One of the *ilv* mutations, which was not suppressed by *supH11*, *ilv-188*, is known to be an *ochre* mutation. In addition it was shown that neither of the two *ochre* mutations *his-4* and *try-3* were suppressed by *supH11*. In the same study it was shown that none of four different ochre suppressors, *supL*, *supM*, *supN*, *supO*, suppressed the *ilv-145* mutation.

Suppression of *amber* mutations of phage T4 by *supH25* was tested using

TABLE 6

Characteristics of secondary revertants of GE143

Secondary revertant (isolation number)	Ilv phenotype at 37°C	Presence of *supH25**	Secondary reversion at *supH* locus
A1	—	no	yes
A2	—	no	yes
B3	+	yes	no
B4	+	yes	no

* Tested as described in text.

strains GE196 and GE199 as indicators. The following *amber* mutants were tested, none of which was found to be suppressed: N67, N102, N120, N128, N131, N132, B255. Previously, the *amber* mutants N130 and N135 had been tested for suppression by *supH11*, also with negative results (EGGERTSSON and ADELBERG 1965).

To test whether the *ilv-145* mutation is suppressible by *amber* suppressors, this mutation was introduced by co-transduction with *metE*$^+$ into *metE46* carrying derivatives of strains S26R1E, S26R1D and H12R8a which carry the Su-1$^+$ Su-2$^+$ and Su-3$^+$ *amber* suppressors, respectively (GAREN, GAREN and WILHELM 1966). The *ilv-145* mutation was not suppressed in any of these strains.

Taken together, these results suggest that the *supH* suppressors belong to neither the *ochre* nor the *amber* class of nonsense suppressors.

Suppression of lac-341 *by* supH25: To determine whether mutations which are suppressible by *supH* suppressors may occur outside the *ilv*-region, mutations of the *lac* operon have been tested for suppression by such suppressors. A total number of 440 *lac* mutations which were induced by EMS in strain GE196 (carrying *ilv-145*) were scored for concomitant reversion by EMS to the Lac$^+$ and Ilv$^+$ phenotypes. Such reversion was found in the case of only one of these mutations, *lac-341*. The appropriate tests showed that *lac-341* is suppressed by *supH25*.

DISCUSSION

Inhibitory effects of suppressor mutations on bacterial growth have previously been observed by several workers (APIRION 1966; BECKWITH 1964; EGGERTSSON and ADELBERG 1965; GARTNER and ORIAS 1965). Such effects are not unexpected in the light of current theories of suppressor action according to which suppressors cause alterations in the specificity of tRNA molecules (CAPECCHI and GUSSIN 1965; ENGELHARDT, WEBSTER, WILHELM and ZINDER 1965; CARBON, BERG and YANOFSKY 1966; GUPTA and KHORANA 1966) or of other components of the protein synthesizing system (for a review see GORINI and BECKWITH 1966). Thus, errors in translation, e.g. caused by an altered tRNA species, might lead to the production of non-functional proteins to the extent that a reduction in growth rate would follow. In the case of the *supH* suppressors, there is no direct evidence that they act at the translational level. This, however, has been our working hypothesis, and the finding that mutations in two unrelated operons, *ilv* and *lac*, are suppressed by *supH25* provides indirect evidence for its validity. It has further been assumed that in strains carrying *supH* suppressors, errors in translation which at low temperature (e.g. 30–38°C) cause suppression and some reduction in growth rate, will be increasingly frequent as the temperature is raised from 38° to 43°C. A resulting production of nonfunctional proteins is, according to this hypothesis, responsible for the inhibitory effects on macromolecular synthesis (DNA, RNA, protein), cell growth, and bacteriophage multiplication observed at high temperatures with cells carrying *supH* (e.g. *supH25*).

Multiplication of phage T4 in cells carrying *supH25* was found to be strongly inhibited at 43°C even when expression of phage functions was allowed to take

place at 37°C for 10–15 minutes before a shift to 43°C. These results indicate that the primary effect of the temperature shift is to alter the function of a bacterial component (e.g. a component of the protein synthesizing system) which is essential for phage multiplication and is existent in the cell prior to phage infection. This component might be the product of the *supH* gene.

A suppressor which causes temperature sensitivity of growth in *E. coli* has been described by APIRION (1966) and inferred to act at the ribosomal level. This suppressor differs from the *supH* suppressors both with regard to its map position (which is close to the threonine and leucine loci) and by having bacteriostatic rather than lethal effects at 43°C.

The suppressor specificity tests reported in this paper strongly indicate that the *ilv-145* mutation is not a nonsense mutation of the *amber* or *ochre* classes, and the *supH* suppressors do not suppress mutations of these classes. A third class of nonsense mutations (giving rise to the UGA codon) has recently been described (BRENNER, BARNETT, KATZ and CRICK 1967), but the possibility that *ilv-145* belongs to this class has not been investigated. The *ilv-145* mutation is known to cause deficiency in the enzyme dihydroxy acid dehydrase (DH) whereas the activities of the two other enzymes of the isoleucine-valine biosynthetic pathway, threonine deaminase (TD) and transaminase (TA), which are under the control of the same operon as DH (RAMAKRISHNAN and ADELBERG 1965) were found to be unaffected by the presence of *ilv-145* (D. DUGGAN, personal communication; strain AB2096 was used in these studies). The order of the genes in this operon is the following: operator–*ilvA* (TD) –*ilvD* (DH) –*ilvE* (TA) (RAMAKRISHNAN and ADELBERG 1965). By analogy with other polycistronic systems (NEWTON, BECKWITH, ZIPSER and BRENNER 1965; MARTIN, SILBERT, SMITH and WHITEFIELD 1966; YANOFSKY and ITO 1966) a nonsense mutation in the *ilvD* cistron is expected to be polar, i.e. to cause reduction in the activity of TA, whereas a missense mutation in *ilvD* is expected to be non-polar. However, the strength of the polarity effect caused by nonsense mutations is known to depend roughly on their intra-cistronic position, being relatively weak when they are located close to the operator-distal end of a cistron. The position of *ilv-145* in the TD cistron is not known. The finding of normal activity of TA in strain AB2096 shows that *ilv-145* is not strongly polar but a weak polarity effect of *ilv-145* cannot be excluded on the basis of the available data. The possibility that *ilv-145* is a nonsense mutation has, therefore, not been excluded.

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SUMMARY

Suppressors which map at the *supH* locus in *E. coli* are shown to cause strong inhibition of cell growth and bacteriophage multiplication, particularly at temperatures of 42–43°C. At these temperatures they are lethal to cells carrying them. Certain mutations in the *ilv* and *lac* operons are suppressed by these suppressors which are shown not to suppress *amber* or *ochre* nonsense mutations.

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