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T4 Bacteriophage Mutants Suppressible by a Missense Suppressor Which Inserts Glycine in Place of Arginine for the Codon AGA

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Phage mutants of T4 have been isolated which can multiply only on *Escherichia coli* strains which contain a missense suppressor which is known to cause the substitution of glycine for arginine in response to the AGA codon. Mutations producing the suppressible phenotype were mapped and shown to occur in six different phage cistrons. Two of the cistrons were concerned with deoxyribonucleic acid synthesis, two were concerned with phage structural components, and two were concerned with functions required for growth in *E. coli* K-12 but not in *E. coli* B. The burst size of the different phage mutants grown on strains carrying the same suppressor was dependent upon the efficiency of suppression, which in turn is known to be dependent upon the glycyl-transfer ribonucleic acid synthetase activity.

Nonsense mutants of phage T4 have proved to be useful for the isolation and characterization of nonsense suppressors in *Escherichia coli* (16, 19, 20). It was the purpose of the present work to isolate conditional lethal mutants of phage T4 which would respond to a specific missense suppressor. It was hoped that such phage mutants would be useful in the isolation and characterization of new missense suppressors.

Missense suppressor system. A large number of mutants in the A gene of tryptophan synthase of *E. coli* are known to result in amino acid substitutions and, as a consequence, inactivation of the enzymatic function of the corresponding α chains (23). One well-studied example, relevant to these studies, is the transition mutation, *trpA36*, in which arginine (codon = AGA) replaces glycine (codon = GGA) at position 210 in the α chain (15, 23, 25). Suppressors of mutation *trpA36*, designated su^{+}_{36}, which map outside the A gene and restore prototrophy to *trpA36* strains, have been isolated and characterized by Brody and Yanofsky (5, 6). As a consequence of the suppressor mutation, a fraction of the α chains made are active, thereby providing the suppressed strain with some functional tryptophan synthase. We now know that suppression of *trpA36* by su^{+}_{36} occurs because an altered transfer ribonucleic acid (tRNA) accepts glycine, and reads the arginine codon AGA, thereby permitting the incorporation of glycine in place of arginine into position 210 in the α chain (7, 8).

It seemed likely that phage mutants which would respond to the bacterial *trpA36* suppressor could be obtained. Such phage mutants would presumably contain mutations formally equivalent to the *trpA36* mutation in *E. coli*. A phage mutation of this type could result in defective phage because of the incorporation of arginine (codon = AGA or AGG) at the mutationally altered position in the phage protein. Phage mutants carrying this type of mutation would be viable on hosts containing su^{+}_{36} if glycine is an acceptable amino acid at the site affected by mutation. Phage mutants of this kind would be expected to exhibit a high degree of specificity; i.e., they should multiply only on hosts carrying su^{+}_{36} and not on cells carrying other missense or nonsense suppressors. One would also expect that mutations of this kind in the phage should occur in any translatable cistron.

MATERIALS AND METHODS

Media. M9 (1) was used as the liquid minimal medium in these studies, and medium E of Vogel and Bonner (22) supplemented with 0.1% Casamino Acids was used as the minimal plating medium. After autoclaving the minimal media, sterile glucose was added to a final concentration of 0.4%. M9 medium was supplemented with 25 μg of L-tryptophan per ml, as indicated. Enriched Hershey broth (21) was used

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as rich medium and will be referred to as EH broth. The composition of L broth has been described (17), as has that of LC medium for P1 transductions (18). All chemicals used in these media were reagent grade. Agar plates for the above media were made by the addition of 1% agar (Difco); 0.65% agar was added to make "soft" plating agar.

Abbreviations. The symbol *trpA36* indicates mutation A36 in the A gene of tryptophan synthase; the identical mutation A23 was used in some cases, but will be designated A36 for clarity. The symbols su^+ and su^- represent the permissive and restrictive alleles of the suppressor gene, respectively; *glyS* is the gene for glycyl-tRNA synthase; $glyS_H$ is the allele for the enzyme with high specific activity and $glyS_L$ is the allele for the enzyme with low specific activity; *xyl* is the gene for utilization of xylose; *trp* and *thi* represent the loci for tryptophan and thiamine biosynthesis, respectively. Trp^- indicates cells auxotrophic for tryptophan.

Bacterial and phage strains. PB59 (W3110 *trpA36* su^+_{36} $glyS_L$) was derived from PB64 (*trpA36* su^-_{36} $glyS_L$) after introduction of the su^+_{36} allele by phage P1 transduction. PB112a (W3110 *trpA36* su^-_{35} $glyS_H$) is a segregant of PB112 (*trpA36* su^+_{30} $glyS_H$) which is Trp^- because it has lost the suppressor (C. Hill, J. Foulds, L. Soll, and P. Berg, *unpublished data*). Strain S/6 (11), a phage T6-resistant smooth colony derivative of *E. coli* strain B, was provided by E. Orias. PB124 (*trpA36* su^-_{36} $glyS_L$ xyl^-) was a spontaneous xyl^- mutant derived from PB64 by penicillin selection and screening on eosine methylene blue (EMB) xylose plates and was kindly provided by W. R. Folk. PB125 is an su^+_{35} strain derived from PB124 by transduction of su^+_{36}. Strains su^-_{36} (PB126) and su^+_{36} (PB127) containing the $glyS_H$ allele were made by transduction of PB124 and PB125, respectively. They differ from their parents only by transduction of the *glyS* character and the selective marker *xyl* (*see* Fig. 1); the *glyS* locus, which determines the activity of glycyl tRNA synthase is closely linked (about 80%) by P1 transduction to *xyl* (4; Berg and Yanofsky, *unpublished data*). In $glyS_L$ strains the efficiency of suppression of *trpA36* [A-protein/CRM-A (6)] is 3 to 5%, whereas in $glyS_H$ strains the efficiency is 20 to 30% (Berg and Yanofsky, *unpublished data*). One lysate of P1 phage grown on PB112 (su^+_{36} $glyS_H$ xyl^+) was used for all the transductions indicated in Fig. 1 except for the production of PB59. An independent suppressor isolate of mutation *trpA36* (su^+_{159}; Hill et al., *unpublished data*), which permits the same type of suppression event as su^+_{36} was donated by C. Hill. The Hfr R^-_{trp} thi^- (5), W3110 *trpA-E*$_{del}$ amber and ochre suppressor strains (24), the UGA suppressor strain CaJ70, W1485, Ymel, and W3110 strains carrying other missense mutations in the *trpA* gene, and their suppressed counterparts, as well as PB59, PB64, and PB112, were all given to us by C. Yanofsky to whom we are deeply grateful.

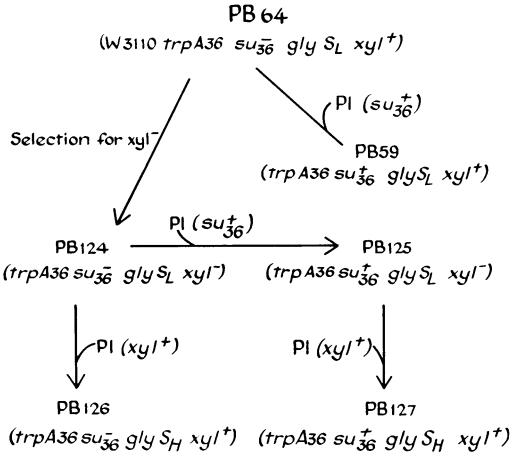

FIG. 1. *Derivation of strains having different efficiencies of suppression.*

The *trpA36* suppressor used in these experiments is derived from suppressor isolate number six described by Brody and Yanofsky (6). Strains carrying su^+_{36} are unstable, and segregate Trp^- (i.e., su^-_{36}) cells at a rate of 1 to 3% in selective (minimal) medium (6; Hill et al., *unpublished data*). Cells were, therefore, prepared for experiments by overnight growth in selective (minimal) medium, and, after dilution of at least 100-fold into the medium of choice, they were allowed to grow for five to six generations. su^-_{36} cells were treated the same way, except that tryptophan was included in the medium for overnight cultures. After growth in the medium of choice, the cells were harvested in exponential growth, concentrated 20-fold by centrifugation, and resuspended in fresh medium.

Phage T4B and multiple amber mutants representing many T4 genes isolated by R. S. Edgar were donated by E. Orias. T4D *r48* and a number of amber mutants of T4 were donated by R. S. Edgar.

Phage techniques. The phage techniques used were those described by Adams (1). Duplicate platings were done for all plaque assays and colony counts. P1 transductions were carried out by the methods described by Luria, Adams, and Ting (18) with the use of P1kc donated by C. Yanofsky.

Mutagenesis of phage. Either strain PB112 or strain PB59 (both su^+_{35}) was used as the host for preparing mutants during mutagenesis of phage stocks. Treatment with 2-aminopurine (2AP) was as described by Edgar and Lielausis (13). Nitrosoguanidine (Aldrich Chemical Co., Milwaukee, Wis.) mutagenesis was performed in tryptone broth as follows. Phage, at a multiplicity of 5, were added to a suspension containing 4×10^8 cells/ml at 37 C in the absence of nitrosoguanidine. After 10 min of adsorption, 10 μliters of a freshly prepared solution of nitrosoguanidine (1 mg/ml) was added to the 1-ml suspension and incubation was continued for 15 min. The mixture was then diluted 10^3-fold and, after 90 min of incubation at 30 C, phage growth was terminated by the addition of chloroform.

Burst-size measurements. Burst size was measured by the procedures of Benzer and Champe (3). Phage

were added to hosts at a multiplicity of 0.01 at 37 C in the medium of choice. For measurements in rich (EH) medium, adsorption occurred for 10 min in the presence of 5×10^{-3} M KCN. After adsorption, T4 antiserum was added to the phage-host mixture for 5 min to reduce unadsorbed phage to a level 0.1% of their original value. Infected cells were diluted at least 10^3-fold into aerated growth medium and, after 45 min of phage growth at 37 C, the cultures were shaken with chloroform and the progeny were plated on the appropriate indicator strain. For measurements in minimal (M9) medium, KCN and T4 antiserum were not used. After 10 min of adsorption, infected cells were diluted 10^3-fold into the growth medium, and phage growth was allowed to occur for 60 min without aeration. At this time, a sample of the infected culture was shaken with an equal portion of twice concentrated EH broth containing chloroform; this mixture was used instead of chloroform alone to minimize surface inactivation of the phage.

Phage crosses. Genetic crosses were performed by a procedure identical with that described above for burst-size measurements, except that each of the two mutants to be crossed was added at a multiplicity of 5 to the strain carrying the suppressor (PB112) at 37 C in EH broth. Control infections with each parental phage alone were routinely performed.

Spot tests. Spot tests were performed by adding one drop (~0.01 ml) of phage stock (at 5×10^7 particles/ml) from a capillary tube to a plate preseeded with 10^8 cells of the appropriate bacterial strain. Complete clearing of the area covered by the drop indicated phage growth; no clearing indicated no phage growth.

Complementation spot tests. The complementation spot test, originally described by Benzer (2), was performed as follows. A 0.5-ml amount of one phage mutant (10^8 particles/ml) was plated with approximately 10^8 cells of strain S/6 on an EH plate. Phage mutants to be tested for complementation with the phage mutant in the bacterial lawn were spotted on the lawn as described for spot tests (*see above*). Since none of the phage mutants used in these tests was able to grow alone on the indicator strain, clearing of the area covered by a spot indicated that complementation had occurred, and the mutations of the phage mutant in the spot and the mutant in the lawn were in different complementation groups. No clearing indicated that the mutations of the two mutants were in the same complementation group. Complementation spots were always compared to control spots on lawns containing no phage. All the phage mutants tested for complementation were used both as phage added with the indicator strain in the lawn and as spots on other bacterial lawns containing phage. In the results reported here, phage mutants gave the same complementation pattern whether they were present in the lawn or were tested as spots against other lawns.

RESULTS

Isolation of phage mutants. Phage mutants were isolated by a modification of the mixed indicator technique of Delbruck (9). Strain PB112a (*trpA36* su^-_{36} $glyS_H$) or strain PB64 (*trpA36* su^-_{36} $glyS_L$) was plated as one layer on an EH plate. This layer was then overlaid with a second layer containing mutagenized phage and the suppressor-containing strain PB112 (*trpA36* $su^{+\ 36}$ $glyS_H$) or strain PB59 (*trpA36 su* ^{36} $glyS_L$), respectively. Wild-type T4 phage grew on either strain and thus produced clear plaques, whereas mutants unable to grow on one of the strains (layers) made cloudy plaques. Cloudy plaques were picked, purified, and spot-tested to detect suppressible phage. Approximately 2×10^5 plaques were screened. Among these, 1 plaque in 10^5 proved to be a suppressible mutant (mutant s3) when strains PB64 (su^{-36} $glyS_L$) and PB59 (su ^{36} $glyS_L$) were used as the screening layers for the mutant isolation. In contrast, when strains PB112a (su^{-36} $glyS_H$) and PB112 (su^{+36} $glyS_H$) were used, 1 in 10^4 plaques yielded suppressible phage mutants. As discussed below, this is probably due to the approximately four- to fivefold higher efficiency of suppression in PB112 compared to PB59. Table 1 shows the parental phage and mutagen used for each phage mutant isolated.

Specificity of the phage mutants for the A36 suppressor. The ability of phage mutants to grow on su^+_{36} and su^-_{36} hosts was tested by spot tests (Fig. 2A) and burst-size measurements (Table 2A). Results of these tests showed that all of the phage mutants grow on su^+_{36} hosts but fail to grow on the su^-_{36} hosts. This is in contrast to the parental T4 phage which is able to grow on both hosts. The reduction in burst size for the mutants on the su^-_{36} compared to the su^+_{36} hosts is approximately 10-fold for s103, but is 30- to 100-fold with each of the others.

The agreement between the burst-size measurements and the spot-test results permitted use of spot tests to determine the specificity of su^+_{36} for suppressing the different phage mutants. Each phage mutant was tested in strains of different genetic backgrounds to determine whether, in each instance, the *trpA36* suppressor was needed for growth and whether other known missense and nonsense suppressors could substitute for su^+_{36} in permitting phage growth (Table 1). It was found that each of the phage mutants fails to grow in any of the genetic backgrounds tested if the host lacks the su^+_{36} allele. The mutants also fail to respond to the presence of either of two other missense suppressors (su^+_{58}, lines 14 and 15; su^+_{78}, lines 16 and 17 in Table 1) or to the amber, ochre, or UGA suppressors tested (lines 18 to 25 in Table 1). These tests, though not exhaustive, indicate that the mutations in the phage, like the *trpA36* mutation

TABLE 1. *Spot tests of phage mutants on various hosts at 37 C on EH plates*[a]

Line	Bacterial hosts[b]			Parental phage, mutagen[c], and phage mutant						
	Laboratory no.	Strain	Genotype	T4B 2AP s3	T4D *r48* 2AP s5	T4B NG s21	T4B NG s22	T4B NG s101	T4B NG s102	T4D *r48* 2AP s103
			trpA36 hosts							
1	PB127	W3110	su^{+}_{36} $glyS_H$	+	+	+	+	+	+	+
2	PB112	W3110		+	+	+	+	+	+	+
3	PB181	W3110(λ)		+	+	+	+	+	+	+
4	PB105	Hfr R^{-}_{trp} *thi*$^{-}$		+	+	+	+	+	+	+
5	PB125	W3110	su^{+}_{36} $glyS_L$	+	+	±	+	−	−	−
6	PB102	W1485		+	+	+	+	−	−	−
7	PB103	Ymel		+	+	+	+	−	−	−
8	PB126	W3110	su^{-}_{36} $glyS_H$	−	−	−	−	−	−	−
9	PB112a	W3110		−	−	−	−	−	−	−
10	PB180	W3110(λ)		−	−	−	−	−	−	−
11	PB44	Hfr R^{-}_{trp} *thi*$^{-}$		−	−	−	−	−	−	−
12	PB124	W3110	su^{-}_{36} $glyS_L$	−	−	−	−	−	−	−
13	PB108	Ymel		−	−	−	−	−	−	−
			Other missense suppressor hosts							
14	PB60	W3110	*trpA58* su^{+}_{58}	−	−	−	−	−	−	−
15	PB57	W3110	su^{-}_{58}	−	−	−	−	−	−	−
16	PB61	W3110	*trpA78* su^{+}_{78}	−	−	−	−	−	−	−
17	PB55	W3110	su^{-}_{78}	−	−	−	−	−	−	−
			Nonsense suppressor hosts							
			trpA-E del							
18		W3110	Ochre su^{+}A	−	−	−	−	−	−	±
19		W3110	su^{+} B	−	−	−	−	−	−	±
20		W3110	su^{+} C	−	−	−	−	−	−	±
21		W3110	Amber su^{+} 1	−	−	−	−	−	−	±
22		W3110	su^{+} 2	−	−	−	−	−	−	±
23		W3110	su^{+} 3	−	−	−	−	−	−	±
24		W3110	su^{-}	−	−	−	−	−	−	±
			trp^{+}							
25	CaJ70	Hfr H	UGA su^{+}_{UGA}	−	−	−	−	−	−	−

[a] The procedure for spot tests has been described in Materials and Methods; + = clearing of spot; − = no clearing of spot; ± = marginal clearing.

[b] Spot tests with PB159 (*trpA36* su^{+}_{159}), which does not grow on rich plates, were performed on medium E plates supplemented with Casamino Acids and tryptophan (Hill et al., *unpublished data*) and gave the same results as the su^{+}_{36} $glyS_H$ strains shown. The following pairs of line numbers represent strains which are isogenic except for the suppressor allele: 1, 8; 2, 9; 3, 10; 4, 11; 5, 12; 7, 13; 14, 15; 16, 17. Strains on lines 18 to 23 are isogenic with the strain on line 24 except for the suppressor allele indicated.

[c] NG = *N*-methyl-*N*-nitro-*N*-nitrosoguanidine; 2AP = 2-aminopurine.

itself, are suppressible only by su^{+}_{36}. We suggest that the mutant codon in each case is most likely AGA.

Response of the phage mutants to different levels of suppression. The efficiency of suppression of *trpA36* in wild-type strains of *E. coli* K-12 can be correlated with two different alleles for glycyl-tRNA synthase (P. Pouwels, P. Berg, and C. Yanofsky, *unpublished data*). One allele ($glyS_L$) is associated with the production of an enzyme with a low specific activity (in extracts) and a high K_m for glycine (2×10^{-3} M); strains carrying the other allele ($glyS_H$) have an enzyme with a high specific activity and a low K_m ($7.5 \times$

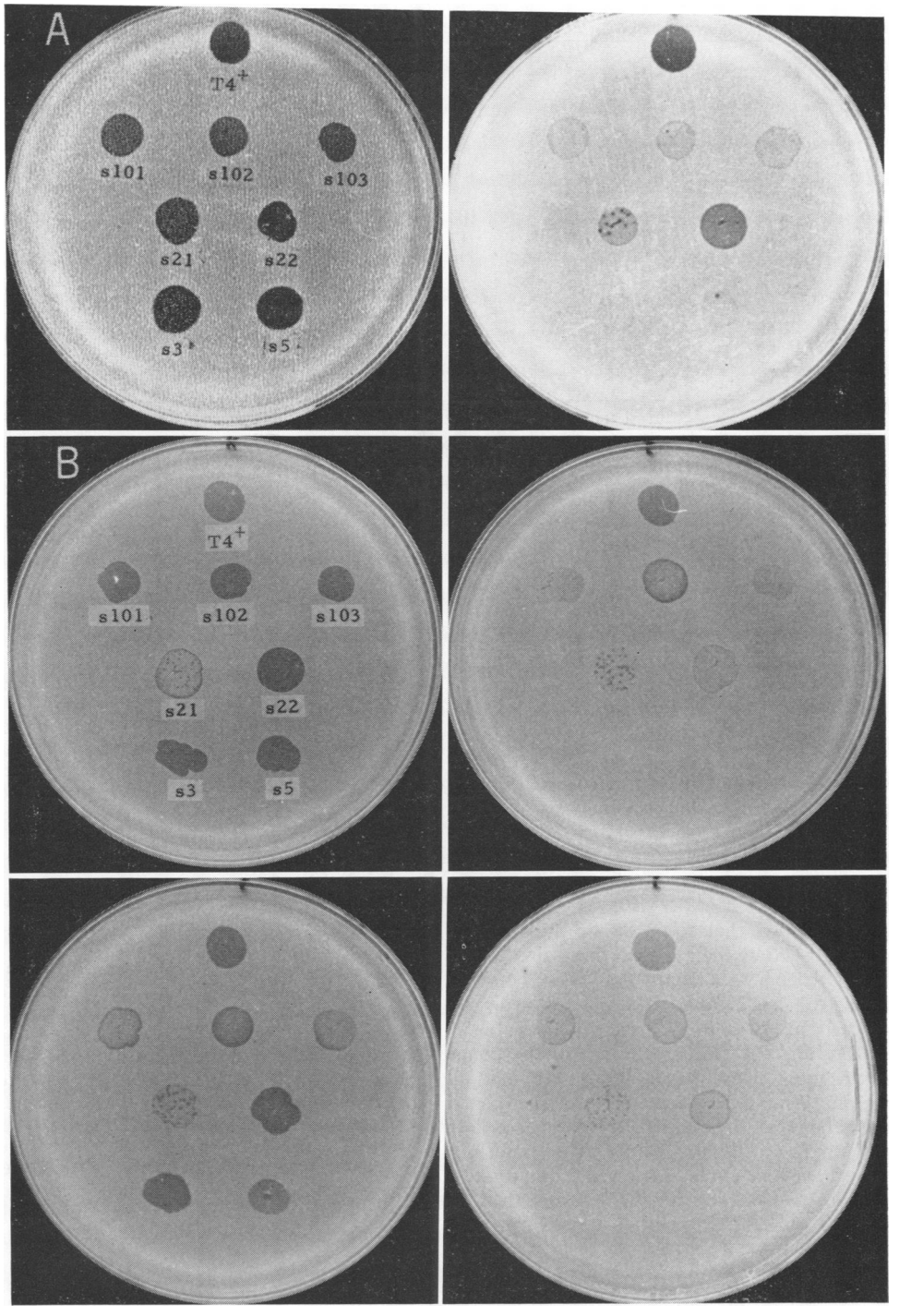

FIG. 2. *Spot tests of the phage mutants and T4. (A) Spot tests on rich (EH) plates. Phage spots as labeled on one plate are the same on the other plate. Bacterial lawns: left, PB127 trpA36 su^{+}_{36} ; right, PB126 trpA36 su^{-}_{36} . (B) Spot tests on minimal (M9) plates containing tryptophan. Phage spots for all the plates are as labeled on the upper left plate. Bacterial lawns: upper left, PB127 trpA36 su^{+}_{36} $glyS_H$; upper right, PB126 trpA36 su^{-}_{36} $glyS_H$; lower left, PB125 trpA36 su^{+}_{36} $glyS_L$; lower right, PB124 trpA36 su^{-}_{36} $glyS_L$.*

TABLE 2. *Burst-size measurements*[a]

Phage	A Avg burst size in EH medium at 37 C		B Avg burst size in M9 medium + tryptophan at 37 C			
	PB127 su^{+}_{36} (expt 1)	PB126 su^{-}_{36} (expt 1)	PB125 *trpA36* su^{+}_{36} $glyS_L$		PB127 *trpA36* su^{+}_{36} $glyS_H$	
			Expt 2	Expt 3	Expt 2	Expt 3
T4+	100 (190)	100 (160)	100 (36)	100 (42)	100 (22)	100 (24)
s3	81	<0.001	17	23	64	67
s5	74	0.1	6.1	12.4	110	133
s21	7.2	0.2	1.1	2.1	3.2	2.7
s22	9.7	0.1	2.0	2.4	5.4	3.6
s101	67	0.3	9.7	5.2	107	62
s102	63	0.3	5.6	5.7	67	56
s103	38	3.4	8.9	8.1	86	89

[a] Burst sizes were calculated using input phage after subtracting unadsorbed phage. All burst sizes were normalized to the burst size of T4B, taken as 100 for each host. The uncorrected burst size of T4B on each host is indicated in parentheses. Experiment numbers indicate that measurements were made in the same experiment.

10^{-5} M) for glycine (4; P. Berg, M. Dieckmann, and J. Kriss, *unpublished data*). As a consequence of the difference in activity of the two enzymes, the ratio of gly-tRNA to $tRNA^{gly}$ found in cells in exponential growth is greater than 0.65 for cells carrying the $glyS_H$ allele and is approximately 0.2 for cells carrying the $glyS_L$ allele (4; Pouwels et al., *unpublished data*). In *trpA36* su^{+}_{36} $glyS_H$ strains, the efficiency of suppression [A/CRM-A(6)] is high (0.2 to 0.3), whereas comparable strains with the $glyS_L$ allele suppress *trpA36* with a lower efficiency (0.03 to 0.05). The difference in the efficiency of suppression probably results from the difference in the rate of esterification of glycine to the suppressor tRNA.

Table 1 shows that some of the mutants can be distinguished by their growth on strains having a high efficiency of suppression. For example, mutant s101, s102, and s103 do not grow on hosts carrying su^{+}_{36} if they are $glyS_L$ (Table 1, lines 5, 6, and 7). The appearance of the phage spots in these tests showed that, although all of the phage mutants except the three mentioned grew on the $glyS_L$ strains, their growth was usually poorer than on $glyS_H$ strains. The burst-size measurements in Table 2*B* give a quantitative measure of the effect of the *glyS* allele; with s101, s102, s103, and s5, there was approximately a 10-fold higher yield on the su^{+}_{36} $glyS_H$ strain than on the su^{+}_{36} $glyS_L$ strain. With s21 and s22, the effect was only twofold, and with s3, about threefold.

Genetic characterization. By performing complementation spot tests between the mutants, it was possible to place all seven phage mutants into six complementation groups. Additional tests with multiple amber mutants of T4 and with amber mutants in which only one gene is affected made it possible to identify the gene affected by the mutation in each of the isolated phage mutants. Table 3 shows the results of complementation tests, the genes assigned, and

TABLE 3. *Parent phage, mutagen used, and gene assignments for the derived phage mutants*

Phage mutant	Phage mutants which fail to complement[a]	Assigned gene	Product of assigned gene[b]
s3	s3, x4b, L91	43	T4 DNA polymerase (10)
s5	s5, x4b, x4c, A14	41	? (DNA negative on Su−) (14)
s21	s21, x4a, B256	5	Tail plate precursor (12)
s22	s22, x4a, B16	7	Tail plate precursor (12)
s101	s101, s102	—	—
s102	s101, s102	—	—
s103	s103	—	—

[a] Mutant x4a has amber mutations in genes 5, 6, 7, and 8; x4b has amber mutations in genes 41, 42, 43, and 44; x4c has amber mutations in genes 41, 44, 46, and 47. B256 has an amber mutation in gene 5; B16, an amber mutation in gene 7. L91 and A14 have temperature-sensitive mutations in genes 43 and 41, respectively. Spot tests on the temperature-sensitive mutants L91 and A14 were performed at 42 C by the procedure described in Materials and Methods. Phage which would be predicted to complement from this table were found in all cases to complement each other.

[b] Products of the assigned genes were taken from the references shown in parentheses.

the products of the assigned genes for some of the phage mutants. No gene assignments were made for s101, s102, or s103. These mutants (as will be shown) grow on strain B of *E. coli*, but not on K-12 strains unless they possess the su^{+}_{36} allele. As such, they are phenotypically equivalent to azure mutants of T4 which grow on strain B but not on K-12 strains; such mutants map in one of two genes close to the rII locus on the T4 map (R. Huskey, *personal communication*). Table 3 shows that the mutation of s103 is in a gene different from the gene affected by the mutations of s101 or s102. Genetic crosses established that s101 and s102, although in the same gene, are different mutations. Crosses also showed that two representatives of s21 had been isolated. Only one of these (called s21 here) was characterized.

The results presented in Table 3 show that the different mutations affect six different cistrons. The mutation in phage s3 affects an enzymatic function [T4 deoxyribonucleic acid (DNA) polymerase], and the mutation in s5 may also affect an enzymatic function (DNA is not produced in the nonpermissive host). The mutations in s21 and s22 have affected structural proteins, whereas those in s101, s102, and s103 have affected functions required for growth on K-12 but not B strains of *E. coli*. The fact that mutations affecting different functions can be suppressed by su^{+}_{36} is consistent with the expectation that the suppressible mutations should occur for many phage functions.

Response of the phage mutants to different temperatures. The phage mutants were tested for their ability to grow at different temperatures on su^{-}_{36} hosts to see whether any of them were temperature-sensitive, and on su^{+}_{36} hosts to detect temperature sensitivity when suppressed. Table 4 shows the results of spot tests on EH plates at various temperatures. As can be seen, phages s101, s102, and s103 grow on host S/6 (a strain B derivative) at all temperatures and grow at 42 C on the K-12 su^{-}_{36} host. As mentioned earlier, these mutants are phenotypically equivalent to the azure mutants of R. Huskey which appear to affect a gene required for growth on K-12 strains but not for growth on B strains. At 30 or 37 C, these mutants fail to grow on the K-12 strain if it does not carry su^{+}_{36}, but the mutations are suppressible, as shown by their growth on K-12 *trpA36* su^{+}_{36}. The growth of these three mutants at 42 C on the K-12 *trpA36* su^{-}_{36} strain has not been examined further. Phage s22 has a temperature-sensitive mutation, since it grows on all hosts at 30 C but fails to grow at higher temperatures except on the su^{+}_{36} strain. This mutant is still slightly temperature-sensitive on the su^{+}_{36} strain, as indicated by a cloudy spot on this host at 42 C. Phages s21 and s5, which fail to grow at any temperature on su^{-}_{36} strains, grow at 30 and 37 C on the su^{+}_{36} strain but do not grow on the su^{+}_{36} host at 42 C. An analysis of 40 temperature-insensitive revertants picked from a plating of phage s21 on the su^{+}_{36} host at 42 C showed that they had all lost the suppressible mutation. While not decisive, this finding is consistent with the idea that it is the suppression of the phage mutation which causes temperature sensitivity. This implies that the suppressor-induced substitution of glycine for arginine in the mutant protein, although it restores function at 30 and 37 C, does not result in an active protein at 42 C. Moreover, since wild-type T4 grows well on this host at 42 C, it follows that the wild-type amino acid at the mutant locus is probably not glycine.

Mature particles of each of the phage mutants were also tested for their sensitivity to heat. Each phage suspension was diluted 100-fold into EH broth at 62 C. At various times thereafter, surviving phage were assayed after diluting

TABLE 4. *Spot tests of the phage mutants and wild-type T4 at three different temperatures*[a]

Phage	Phage mutants																						Wild-type T4		
	s3			s5			s21			s22			s101			s102			s103					
	30 C	37 C	42 C	30 C	37 C	42 C	30 C	37 C	42 C	30 C	37 C	42 C	30 C	37 C	42 C	30 C	37 C	42 C	30 C	37 C	42 C	30 C	37 C	42 C
PB112 su^{+}_{36}	+	+	+	+	+	−	+	+	−	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+
PB112a su^{-}_{36}	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	+	−	−	−	+	+	+
S/6 su^{-}_{36}	−	−	−	−	−	−	−	−	−	±	−	−	+	+	+	+	+	+	+	+	+	+	+	+

[a] Phage were spot-tested on EH plates by the procedure described in Materials and Methods; + = clearing of the area covered by the spot, − = no clearing, ± = marginal clearing.

samples 100-fold into EH broth at 0 C. It was found that only particles of s21 and s103 were significantly affected in comparison with wild-type T4 particles (Fig. 3). The curve for s21 particles suggests a single heat-sensitive target. The multihit curve for s103 particles may indicate that a subunit structure of this phage has been altered by the mutation.

Examination of affected gene products. The complementation tests (Table 3) show that the mutation in phage s3 is in gene 43, the gene for T4 DNA polymerase (10). Assays for DNA polymerase activity were performed in crude extracts of phage-infected su^-_{36} (PB112a) and su^+_{36} (PB112) hosts. Either wild-type T4B or the mutant phage s3 was added at a multiplicity of five at 37 C in L broth, and extracts prepared by sonic treatment were assayed as previously described (10). The enzyme activity for phage s3 on the su^-_{36} host is <1% of that for T4B on the same host. In the cells carrying su^+_{36}, the enzyme activity of phage s3 is approximately 10 to 15% of that for T4B (Table 5). Thus, the presence of su^+_{36} increases the production of functional T4 DNA polymerase more than 20 times.

Phage s21 possesses a mutation in gene 5 (Table 3) which alters a base-plate precursor protein so that noninfective phage are produced in a nonpermissive host. If this mutation in the phage is a missense mutation, as we presume, it follows that noninfective phage are produced from nonpermissive hosts because of the amino acid substitution in the mutationally affected protein. A lysate of this phage, from the nonpermissive host S/6, was examined in the electron microscope to see whether the noninfective phage were complete or incomplete particles. Approximately 400 particles were examined. Four of the particles seen were complete phage; all of the other particles were empty heads. This result is similar to the results obtained when an amber mutant, with a mutation in gene 5, was grown on a nonpermissive host (12). The similarity of the results obtained with phage s21 and the results obtained when phage with amber mutations in the same gene were used may indicate that the mutationally affected base-plate protein in phage s21 interferes with some process in the assembly of phage tails.

TABLE 5. *T4 DNA polymerase activity of phage s3 on su^+_{36} and su^-_{36}*[a]

Phage	Hosts			
	PB112 su^+_{36}		PB112a su^-_{36}	
	Activity	Normalized	Activity	Normalized
T4B	1.8	100	1.7	94
s3	0.21	11.7	<0.02	<0.6

[a] T4 DNA polymerase was assayed by the method of deWaard et al. (10). Enzyme activity is presented both as actual units of enzyme activity per milligram of protein measured (activity) and as activities normalized to the activity for T4B on the PB112 host taken as 100 (normalized). One unit of enzyme activity has been defined (10) as the amount of enzyme necessary for the incorporation of 10 nmoles of ^{32}P-deoxyadenosine triphosphate into an acid-insoluble product in 20 min at 25 C.

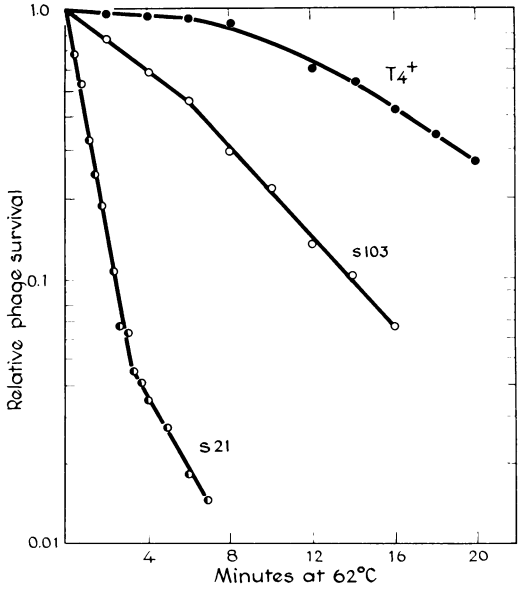

FIG. 3. *Heat inactivation of mature phage particles. The experiment was performed as described in the text. Mature particles of the phage mutants (except for s21 and s103 shown above) exhibited inactivation kinetics similar to that shown for T4.*

DISCUSSION

The data presented here indicate that conditional lethal mutants of bacteriophage T4 can be isolated which are suppressible by a missense suppressor in the bacterial host. As expected, this type of mutation can occur in different phage genes. Therefore, su^+_{36} is not a suppressor for only a single mutated gene; more likely, it suppresses a particular mutant codon which can arise in any gene and presumably from any of several codons. Given the similarity of the suppression pattern of the phage mutations to that of the bacterial mutation *trpA36*, one would

predict that the phage mutations are missense mutations which give rise to AGA (or possibly AGG) in the messenger RNA and, as a consequence, produce complete but functionally defective protein in nonpermissive hosts.

The wild-type codon which gives rise to the suppressible codon has not been identified. It could be GGA (or GGG), which corresponds to glycine; in that case, suppression restores the wild-type amino acid. The fact that, on su^{+}_{36} hosts, the phage mutants s5 and s21 do not grow at 42 C, whereas wild-type T4 does, indicates that the suppressed proteins are temperature-sensitive and, therefore, different from the corresponding wild-type proteins. This could argue that in these two cases the wild-type amino acid was not glycine and that insertion of glycine, although it restores function, makes the protein temperature-sensitive. Alternatively, the wild-type amino acid *is* glycine. In this case, temperature sensitivity is observed because either (i) the mutant protein activity is temperature-sensitive and is required for growth in addition to the small fraction of suppressed (wild type) activity, or (ii) the affected protein is a subunit, and the combination of mutant and suppressed (wild type) subunits, while active, is temperature-sensitive.

The results of burst size measurements on two different su^{+}_{36} hosts (Table 2B) show that most of the phage mutants grow better on strains which are $glyS_H$ than on $glyS_L$. This correlates with the increased efficiency of suppression of *trpA36* in $glyS_H$ strains compared to $glyS_L$ (Pouwels et al., *unpublished data*).

The phage mutants described here were isolated from mutagenized phage with a frequency of 10^{-4} when the double layer used for detecting mutants contained PB112 (su^{+}_{36} $glyS_H$) and PB112a (su^{-}_{36} $glyS_H$), i.e., when the efficiency of suppression in the permissive host was high. The frequency of detecting mutants was approximately 10 times lower when the permissive host in the double layer was su^{+}_{36} $glyS_L$. It is very likely that mutants which require considerable suppression to grow would not have been seen in the latter case. This suggests that this approach to the isolation of missense mutants of phage suppressible by weak suppressors (e.g., 1% or less) would be laborious. With weak suppressors, one might expect to find suppressible mutants with the only genes affected being those which code for functions needed in small amounts, e.g., enzyme, and not in genes which code for phage structural proteins.

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