JOURNAL OF BACTERIOLOGY, Mar. 1982, p. 1082–1088
0021-9193/82/031082-07$02.00/0

Vol. 149, No. 3

Evidence That the Two *Escherichia coli groE* Morphogenetic Gene Products Interact In Vivo

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Received 23 June 1981/Accepted 12 October 1981

The *Escherichia coli groEL* and *groES* gene products are essential for both phage morphogenesis and bacterial growth. Although the gene products have been identified, their exact roles in these processes are not known. We have isolated mutations in the *groEL* gene that suppress defects in the *groES* gene. These intergenic suppressors were shown to map in the *groEL* gene by a variety of genetic and biochemical analyses. These results suggest that the two morphogenetic gene products interact in vivo and help to explain why mutations in either gene exhibit the same phenotype with respect to λ head assembly and bacterial growth.

Although *Escherichia coli groE* mutants were isolated as hosts in which bacteriophage λ is unable to undergo productive infection, such mutants were subsequently shown to be pleiotropic, also affecting the growth of phages T4 and T5 and the bacterial host itself (2, 3, 12, 13). All of the effects on phage growth seem to be at the level of morphogenesis. Bacteriophages λ and T4 are unable to make functional heads in *groE* hosts, whereas T5 makes heads normally but only undergoes abortive tail assembly (16). The *groE* gene products must be necessary for bacterial growth, since many *groE* mutants are temperature sensitive for bacterial growth, forming long filaments without septa at 42°C (1).

λ *groE*$^+$ transducing phage have been isolated from pools of *Eco*RI and *Hin*dIII fragments of *E. coli* DNA ligated into various phage vectors (4, 6, 8). Such phage are able to form plaques on all known *groE* bacterial mutants, indicating that they carry all *groE* genes in their bacterial DNA (4). Analysis of point and deletion mutations of these phage has led to the identification of two *groE* genes and their products (4, 6, 15). One gene product (designated gp*groEL*, since it is the larger *groE* gene product) is a protein of 65,000 M_r which has been purified to homogeneity (5, 7). In its native state gp*groEL* is a decatetramer, with its 14 subunits arranged in a double ring with sevenfold symmetry. This complex sediments at 25S and has a weak ATPase activity (5, 8). gp*groES* has recently been shown to be a protein of 15,000 M_r (15).

Genetic studies have helped to clarify the position at which the *groE* gene products act in phage morphogenesis. λ mutants able to overcome the *groE* block (called λε) arise at a frequency of 10^{-7} to 10^{-8} and have mutations in either the *B* or the *E* gene (2). The λ *E* gene mutants seem to lower the levels of active gp*E* in the infected cell, allowing the residual activity of a mutant gp*groE* to complete a few λ heads. Murialdo (8) has shown by sedimentation experiments that at least gp*groEL* interacts with gp*B* at a very early stage in λ head assembly.

Out of 11 *groEL* mutants in our collection, only 1, mutant *groEL44*, does not allow T4 growth. In addition, we found that six out of six *hd* bacterial mutants of Revel et al. (11), which block T4 head assembly at the level of gp*31* action, map in the *groEL* gene (unpublished results). These findings suggest that gp*groES* may be nonessential for T4 head assembly.

The identification of a second *groE* gene raises the possibility of cooperation between the two *groE* gene products. The λ proheads formed upon infection of bacteria with mutations in either *groE* gene have a similar composition, suggesting that gp*groES* may act at the same step as gp*groEL* in λ head morphogenesis (15). In this paper we describe genetic and biochemical experiments indicating that the proteins interact in vivo, suggesting that the two proteins act concomitantly, rather than sequentially.

MATERIALS AND METHODS

Phage and bacterial strains. *E. coli groE* mutants derived from B178, all of which have the same phenotype with respect to λ growth, have been shown to fall into two complementation groups (15). The *groES619* strain is a representative of one of these groups, carrying a mutation in the gene encoding the 15,000-M_r *groE* polypeptide which prevents normal λ head assembly and makes the bacterium temperature sensitive for growth. T4 grows normally in strain *groES619* and all other *groES* mutants. The *groEL44* strain has a

mutation in the *groE* gene encoding the 65,000-M_r protein, blocks λ head morphogenesis at the same step as strain *groES619*, and is also temperature sensitive for bacterial growth. T4 does not make functional heads in *groEL44* bacteria (3). The construction of λ *groE*$^+$ transducing phage carrying either or both of the *groE* genes has been previously described (4, 8, 15). T4ε mutants are T4 derivatives able to bypass the *groE* block found in a specific *groEL* mutant [i.e., T4ε1 was selected for growth on strain *groEL44*, whereas T4ε3 was selected for growth on strain *groES619*(Ts$^+$)*4*].

Isolation of temperature-resistant revertants. Temperature-resistant revertants of *groES619* were isolated by incubating *groES619* bacteria on T plates at 42°C for 2 days and were obtained at a frequency of about 10^{-7} to 10^{-6}.

Labeling experiments. Bacteria to be labeled were grown overnight at 30°C in high-sulfur M9 medium (4) supplemented with all amino acids except methionine and cysteine. They were diluted 1/10 into the same medium, grown until the cultures had reached a concentration of 3×10^8 bacteria per ml, and shifted to 42°C for 10 min. They were then labeled with 20 μCi of [^{35}S]methionine per ml (1,380 Ci/mmol, Amersham SJ204) for 10 min at 42°C. The labeling was stopped by centrifugation, and the pellets were resuspended in 0.05 volume of two-dimensional lysis buffer (10). T4 proteins were labeled by infecting log-phase (3×10^8/ml) cultures of the bacteria of interest with T4 at a multiplicity of infection (MOI) of 10, growing 20 min at 37°C with aeration, and labeling with 80 μCi of [^{35}S]methionine per ml for 10 min. The cultures were centrifuged for 1 min at 15,000 × *g*, and the pellets were resuspended in 0.20 volume of sodium dodecyl sulfate sample buffer.

One- and two-dimensional polyacrylamide gel electrophoresis. These were as previously described (15).

Complementation test with T4 phage mutants. About 2×10^8 *groEL140 sup*$^+$ bacteria were mixed with 10^8 T4 am*NG71* (or another T4 amber or ε mutant), 3 ml of soft agar was added, and the mixture was plated on L plates. Approximately 20 μl of various phage suspensions (containing about 3×10^6 phage) was placed on top of the soft agar and allowed to dry. After overnight incubation at 37°C, positive complementation resulted in the confluent lysis of the bacterial lawn at the site of the drop, whereas negative complementation resulted in few or no plaques. The results of spot complementation tests were occasionally verified by measuring phage yield after coinfection of liquid cultures of *groEL140 sup*$^+$ bacteria with an MOI of 10 of each T4 mutant strain.

Media and bacterial and phage platings. These were as previously described (2, 4).

Phage yield experiments. The effect of preinfection by various λ *groE* transducing phage on T4 infection was determined by phage yield experiments. The bacteria to be tested were grown to 3×10^8/ml, and various λ phage were added at an MOI of 10. After 20 min of adsorption at room temperature, the cultures were shaken at 37°C for 30 min. T4 or T4ε1 was then added at an MOI of 5, and the cultures were shaken at 37°C for 10 min. The cultures were spun for 1 min at 15,000 × *g*, and the supernatants (containing unadsorbed phage) were discarded. The pellets were resuspended in 0.1 ml of L broth and shaken another 50 min at 37°C. A few drops of $CHCl_3$ were added to complete lysis, and the T4 progeny were assayed on *E. coli* B, on which host phage λ does not form plaques.

RESULTS

Isolation of suppressors of *groES619*. Experiments were designed to determine whether the apparent concerted action of the *groE* gene products involved protein-protein interactions between them. We reasoned that if we could suppress mutations in one gene by mutating the second gene, this would support this hypothesis. The only *groE* mutants shown to block T4 growth (some of which permit λ growth [11]) map in the *groEL* gene (unpublished data), so this distinguishing phenotype was used for selecting altered *groEL* genes. The strategy used for isolating specific intergenic suppressors was to start with *groES* mutants which are temperature sensitive for bacterial growth, isolate temperature-resistant derivatives, and screen those survivors for inability to propagate phage T4 or T4ε1 (a T4 mutant able to grow on strain *groEL44*). Both T4 and T4ε1 propagated normally on all *groES* mutants tested.

Several classes of temperature-resistant bacteria were obtained. Strain *groES619*(Ts$^+$)*1* is a representative of the first and most populous class, isolated at a frequency of 5×10^{-7}. These bacteria now permit the growth of all λ and T4 derivatives (Table 1), and they probably represent true revertants of the *groES619* mutation. Strains *groES619*(Ts$^+$)*7* and (Ts$^+$)*10* are representatives of the second class, found about 20-fold less frequently than the revertants in the first class. On these hosts, λ grows normally, T4 makes small plaques at a close to wild-type efficiency, and T4ε1 forms plaques at a frequency of less than 10^{-4} (Table 1). The third class [e.g., *groES619*(Ts$^+$)*4*] does not allow the growth of T4 or T4ε1, while permitting λ to grow normally. Since the last two classes of temperature-resistant revertants have the phenotype of *groEL* mutants, they were subjected to further analysis.

The second and third types of temperature-resistant revertants (putative *groEL* mutants) were isolated from strains *groES619* and *groES42* at a frequency of 1 to 5% of all temperature-resistant revertants, but not from strain *groES7* (although at least 1,000 temperature-resistant revertants were tested). The frequencies with which temperature-resistant bacteria were obtained in all cases were comparable, so the mutations required to give such a phenotype must be allele dependent.

Isolation of T4ε mutants on temperature-resistant hosts. When T4 phage were plated on the *groES619*(Ts$^+$) mutants that were not true revertants, plaques were formed at a frequency of

TABLE 1. Plating properties of various phage on *groE* strains

Bacterial strain	Efficiency of plating[a] of phage strain:						
	λ	λ *groES*⁻ *groEL*⁻	λ *groES*⁺ Δ(*groEL*)	λ Δ(*groES*) *groEL*⁺	T4	T4ε1	T4ε3
B178 *groES*⁺ *groEL*⁺	+	+	+	+	+	+	+
groEL44	−	+	−	+	−	+	−
groEL140	−	+	−	+	+	+	−
groES619(Ts)	−	+	+	−	+	+	+
groES619(Ts⁺)*1*	+	+	+	+	+	+	+
groES619(Ts⁺)*4*	+	+	+	+	−	−	+
groES619(Ts⁺)*4* (λ *imm*²¹ *groES*⁺ *groEL*⁺)	+	+	+	+	+	+	+
groES619(Ts⁺)*7*	+	+	+	+	±	−	+
groES619(Ts⁺)*7* (λ *imm*²¹ *groES*⁺ *groEL*⁺)	+	+	+	+	+	+	+
groES619(Ts⁺)*10*	+	+	+	+	±	−	+
groES619(Ts⁺)*10* (λ *imm*²¹ *groES*⁺ *groEL*⁺)	+	+	+	+	+	+	+

[a] (+) Denotes large plaque size and an efficiency of plating of 0.5 to 1.0 at 37°C when the efficiency of plating on B178 is taken to be 1.0. (±) Denotes an efficiency of plating of 0.1 to 1.0 with small plaques, and (−) denotes an efficiency of plating of less than 10^{-4}.

approximately 10^{-6}. One of these [T4ε3, isolated on strain *groES619*(Ts⁺)*4*] was grown up and tested on various *groE* and temperature-resistant revertant strains (Table 1). Since T4ε3 was found to be unable to grow on *groEL140* bacteria, on which T4 wild type can grow, this bacterial host was used for mapping the position of the ε mutation. In complementation tests on *groEL140 sup*⁺ bacteria, T4ε3 was able to complement phage bearing amber mutations in all genes tested except gene *31*, placing the mutation in that gene. The result shows that the inability of phage T4ε3 to propagate on strain *groEL140* is at the level of gp*31* action. Since a mutation in gene *31* can overcome the block on T4 growth found in *groES619*(Ts⁺)*4* bacteria, it is likely that T4 cannot grow because wild-type gp*31* is inactive in this host.

Phenotype of T4 infection. T4 infection of *groEL44* bacteria is blocked at the level of gp*31* action, resulting in the absence of the proteolytic cleavages found in normal head assembly (3) (Fig. 1, lane 13). The protein profiles after T4 infection of *groES619*(Ts⁺) derivatives were compared with those in strains *groEL44* and *groES619* to determine the position of the block in T4 growth. The proteins synthesized in *groEL44*, *groES619*, and two temperature-resistant revertants [(Ts⁺)*4* and (Ts⁺)*7*] were labeled between 20 and 30 min after infection with T4 (or its derivatives T4ε1, T4ε3, or T431⁻ [am*NG*71]). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the proteins revealed that the pattern of gp*23* cleavage exactly parallels the pattern of phage growth; gp*23* (and two other proteins, which have not been identified) is not cleaved when the phage-host combination is nonproductive (Fig. 1). There are no differences between the protein profiles found in T4-infected *groEL44* bacteria and T4 am*NG*71-infected *groES619* bacteria, in which cases the blocks have been proven to be at the level of gp*31* function, and those found after T4 infection of *groES619*(Ts⁺) derivatives. These results suggest that strains *groES619*(Ts⁺)*4* and (Ts⁺)*7* block T4 and T4ε1 phage growth at the level of action of gp*31*.

Lysogenization experiments. The third step in analyzing the *groES619*(Ts⁺) revertants was to lysogenize them with λ *imm*²¹ *c*I⁺ transducing phage bearing both of the *groE* genes. The results of the lysogenization experiments are presented in Table 1. In all cases, lysogenization with a λ *imm*²¹ *c*I⁺ *groES*⁺ *groEL*⁺ transducing phage made the strain wild type with respect to both λ and T4 growth. This is because the wild-type genotype is dominant over the mutant alleles, and the prophage copies of the *groE* genes are expressed at the same levels as the bacterial copies (see Fig. 2) (4).

T4 phage yield experiments. We determined T4 phage yield in various bacterial strains both with and without preinfection by various λ *groE* transducing phage. Similar results were obtained when *groES619*(Ts⁺)*4* bacteria were infected with T4 or *groES619*(Ts⁺)*10* bacteria were infected with T4ε1 (Table 2). In these cases, the yield of T4 or T4ε1 was reduced by a factor of 100 to 500 from that found in either B178 *groES*⁺ *groEL*⁺ or *groES619*(Ts). Preinfection with λ *imm*²¹ *c*I⁺ or λ *imm*²¹ *c*I⁺ *groES*⁺ Δ(*groEL*) for 30 min had no effect on the T4 or T4ε1 phage yield. Preinfection with either λ *imm*²¹ *c*I⁺ *groES*⁺ *groEL*⁺ or λ *imm*²¹ *c*I⁺ Δ (*groES*) *groEL*⁺, however, had the effect of increasing the T4 or T4ε1 phage yield by a factor of 100 to 200. In this series of experiments, the presence of wild-type gp*groEL* suppressed the

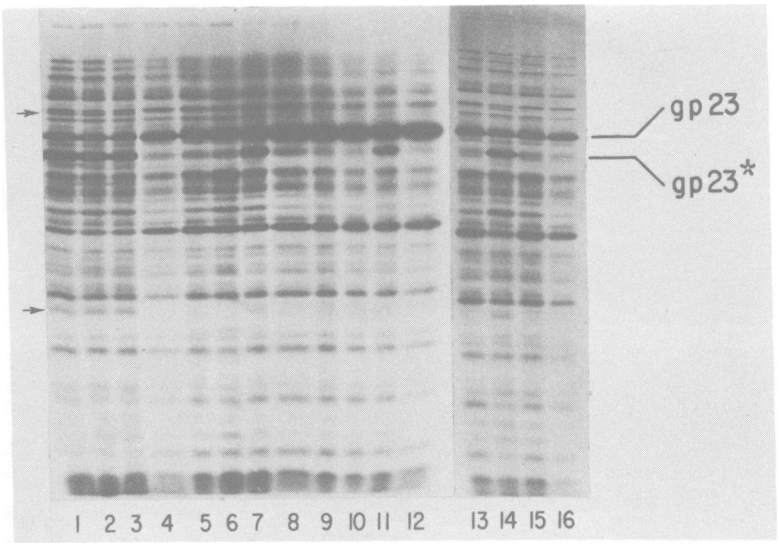

FIG. 1. Autoradiograms of sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis. Various hosts were infected with phage T4 (lanes 1, 5, 9, 13), T4ε1 (lanes 2, 6, 10, 14), T4ε3 (lanes, 3, 7, 11, 15), or T4 am*NG71* (31$^-$) (lanes 4, 8, 12, 16) and labeled with [^{35}S]methionine at 40 μCi/ml from 20 to 30 min after infection. The bacteria were *groES619* (lanes 1 to 4), *groES619*(Ts$^+$)*4* (lanes 5 to 8), *groES619*(Ts$^+$)*7* (lanes 9 to 12), and *groEL44* (lanes 13 to 16). The arrows mark the positions of two unidentified proteins that are only seen when gp*23* is cleaved. The acrylamide was 12.5%.

block on T4 or T4ε1 growth, and supplying λ proteins alone or gp*groES* plus λ proteins was not sufficient to compensate.

Biochemical analysis. Because the phenotype of the revertants, the lysogenization experiments, and the phage yield results all pointed to the *groEL* gene as the site of the suppressor mutations, some of these mutations might be expected to alter the characteristics of the *groEL* polypeptide. Since gp*groEL* is known to be synthesized at a relatively higher rate after a shift from 30 to 42°C (9; D. Drahos and R. W. Hendrix, J. Bacteriol., in press; unpublished data), bacteria were labeled with [^{35}S]methionine between 10 and 20 min after such a shift to increase its molar amount in the cell. The bacterial proteins made during such a pulse were displayed on two-dimensional polyacrylamide gels. Strain *groES619*(Ts$^+$)*7* and 10 other strains were shown to encode gp*groEL* with a wild-type isoelectric point (Fig. 2b). However, strain *groES619*(Ts$^+$)*4* and two other strains were shown to possess gp*groEL* with a more basic isoelectric point (Fig. 2c). The magnitude and direction of this shift are most apparent when (Ts$^+$)*4* bacteria lysogenic for λ *groES*$^+$ *groEL*$^+$ are heat shocked and labeled (Fig. 2d). In this case, two spots replace the normal gp*groEL* spot. Because the intensities of the spots are similar, the wild-type protein encoded by the λ transducing phage and the mutant protein from the bacterial chromosome appear to be synthesized in approximately equal amounts. Since such a lysogen regains the wild-type phenotype (Table 2), the mutation must be recessive. The discovery of a spontaneous change in the biochemical properties of gp*groEL* coincident with the genetic results confirm our hypothesis that a mutation in the *groEL* gene can suppress the phenotype of a mutation in the *groES* gene.

DISCUSSION

The evidence that the *groEL* and *groES* gene products interact stems from the isolation of bacteria in which a *groEL* mutation has suppressed a *groES* mutation. The suppressor mutations, which make strain *groES619* temperature resistant for growth at 42°C, are in the *groEL* gene by four criteria. (i) The phenotype of the temperature-resistant revertants is that of *groEL* mutants. T4ε1 and, in some cases, T4 do not grow, and the proteins normally cleaved during head morphogenesis of these phage remain in their uncleaved forms. T4ε3, which is a T4 mutant able to grow on all the *groES619*(Ts$^+$) mutants, has an altered gene *31* protein, showing that the block in T4 infection is at the level of gp*31* action, as is found in known *groEL* mutants. (ii) Lysogenization with a λ *imm*^{21} *groES*$^+$ *groEL*$^+$ phage is sufficient to restore the ability of T4 to grow on temperature-resistant rever-

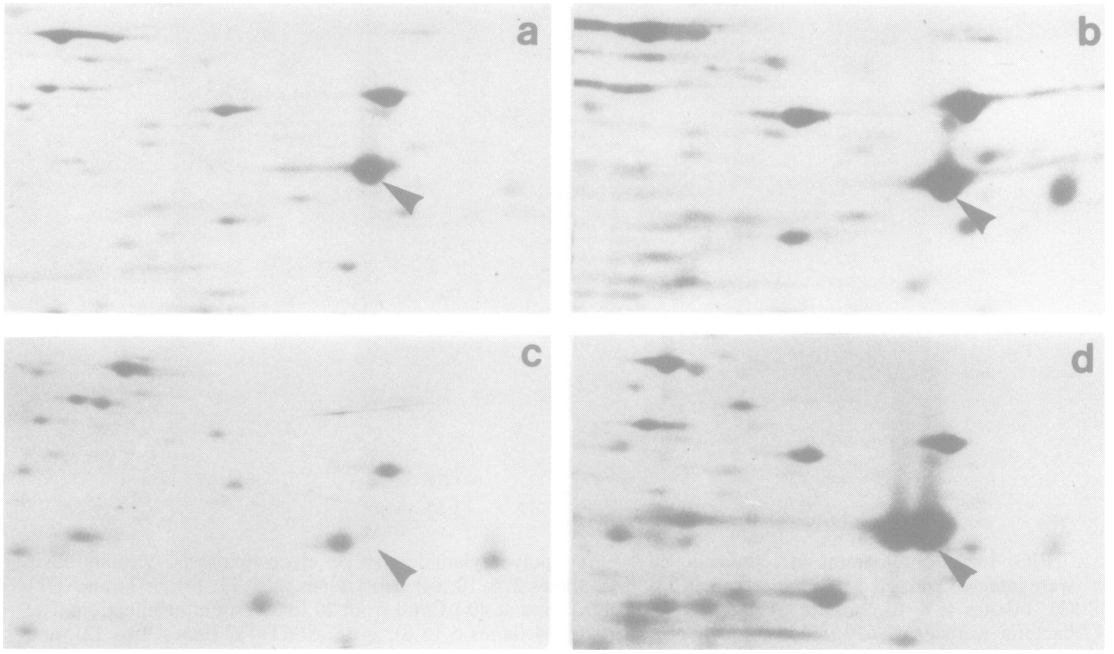

FIG. 2. Two-dimensional gel electrophoresis of bacterial proteins labeled with 20 μCi of [^{35}S]methionine per ml from 10 to 20 min after a shift from 30 to 42°C. The bacteria were (a) *groES619*, (b) *groES619*(Ts$^+$)*7*, (c) *groES619*(Ts$^+$)*4*, and (d) *groES619*(Ts$^+$)*4* (λ *imm*^{21} *groES*$^+$ *groEL*$^+$). Only the regions of the gels corresponding to a 6.0 to 4.5 pH gradient (in the horizontal dimension) and approximately 58,000 to 75,000 molecular weight (in the vertical dimension) are shown. The arrow points to the position of wild-type gp*groEL*.

TABLE 2. T4 phage yield in *groES619*(Ts$^+$) bacteria[a]

Bacterial host	Preinfecting phage	Phage yield	
		T4	T4ε1
B178 *groES*$^+$ *groEL*$^+$	none	99.5	51
	λ *imm*^{21} *cI*$^+$	122.5	148
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ *groEL*$^+$	74	59
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ Δ(*groEL*)	85.5	83.5
	λ *imm*^{21} *cI*$^+$ Δ(*groES*) *groEL*$^+$	64	92
groES619(Ts$^+$)*7*	none	3.4	ND[b]
	λ *imm*^{21} *cI*$^+$	3.6	ND
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ *groEL*$^+$	37.2	ND
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ Δ(*groEL*)	1.5	ND
	λ *imm*^{21} *cI*$^+$ Δ(*groES*) *groEL*$^+$	58.2	ND
groES619(Ts$^+$)*4*	none	0.2	ND
	λ *imm*^{21} *cI*$^+$	0.2	ND
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ *groEL*$^+$	37.2	ND
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ Δ(*groEL*)	0.2	ND
	λ *imm*^{21} *cI*$^+$ Δ(*groES*) *groEL*$^+$	35.4	ND
groES619(Ts$^+$)*11*	none	ND	0.3
	λ *imm*^{21} *cI*$^+$	ND	0.4
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ *groEL*$^+$	ND	29.0
	λ *imm*^{21} *cI*$^+$ *groES*$^+$ Δ(*groEL*)	ND	0.3
	λ *imm*^{21} *cI*$^+$ Δ(*groES*) *groEL*$^+$	ND	27.4

[a] The phage yield experiments were done as described in the text.
[b] ND, Not determined.

tants, whereas lysogenization with λ *imm*^{21} alone is not sufficient. (iii) The reduced T4 phage yield found in temperature-resistant revertants was increased by a factor of 100 to 200 by preinfection with λ *imm*^{21} *groES*$^{+}$ *groEL*$^{+}$ or λ *imm*^{21} Δ(*groES*) *groEL*$^{+}$, whereas preinfection with λ *imm*^{21} or λ *imm*^{21}*groES*$^{+}$ Δ(*groEL*) had no effect. (iv) Of 14 temperature-resistant revertants, 3 synthesized *groEL* polypeptide with an altered isoelectric point. The combination of genetic behavior with the presence of a physically altered *groEL* protein in mutants obtained without mutagenesis places the suppressor mutations in the *groEL* gene. Our ability to isolate such suppressors is strong evidence that the *groE* gene products interact in vivo.

The existence of interaction between gp*groEL* and gp*groES* suggests the possibility of the two proteins acting together at the same step. Action at the same step is consistent with our observations that the abnormal λ proheads found in mutants in either gene are identical (15). There are mutants in both genes that block T5 tail assembly, and there are mutants in both genes that are temperature sensitive for bacterial growth. We have not obtained any evidence that the *groES* protein is required for T4 morphogenesis. There may be more than one active site on a gp*groEL*-gp*groES* complex, one of which is composed of parts of both proteins (and is required for λ, T5, and *E. coli* growth) and one of which consists of only *groEL* protein (and is required for λ, T4, T5, and *E. coli* growth). Changes in either site may not be independent, because the mutants we obtained must affect both. Alternatively, there may be only one active site, but the levels of functional *groE* complex required for each system are different.

It is likely that we have not studied all *groEL* suppressors of the *groES619*(Ts) mutation, since we only analyzed the 1 to 5% of the temperature-resistant survivors that blocked T4 or T4ε1 growth. It is clear that the *groEL* gene can be mutated without affecting T4 growth, since only 1/11 of the *groEL* mutants in our collection (none of which allow λ morphogenesis) block T4 head assembly. Therefore, many more of the survivors that we obtained may carry *groEL* suppressors. In fact, at least three independent temperature-resistant revertants had the same shift in isoelectric point, indicating that we may be analyzing a few specific mutations that are capable of compensating for the *groES619* mutation.

An important observation was that we were unable to isolate temperature-resistant revertants that block T4 growth from some *groES* mutants. These strains may either have mutations that cannot be compensated for by a viable *groEL* mutation (e.g., affecting part of gp*groES* that does not interact with gp*groEL*) or mutations that can be suppressed by *groEL* mutation without affecting the protein's activity in T4 head assembly. The existence of such allele specificity for suppression in *groES* mutants strongly argues against the possibility that two independent mutations cause the temperature-resistant and T4 nonpermissive phenotypes. In addition, spontaneous *groES619*(Ts^{+}) revertants which simultaneously block T4 head morphogenesis represent 1 to 5% of the surveyed population. This frequency is much higher than that at which one would expect to find double mutants in the absence of mutagenesis.

Although these studies suggest that the *groE* proteins act together as a complex in bacterial growth and phage morphogenesis, no biochemical evidence for the existence of such a complex has yet been obtained. The *groES* protein does not sediment with the 25S *groEL* protein decatetramer on glycerol gradients nor does it precipitate with anti-gp*groEL* antibodies (unpublished observations). Further analysis of the nature of the interaction between the two proteins and their function in bacterial and phage morphogenesis must await the fine-structure mapping of specific mutations and in vitro studies on the purified proteins.

ACKNOWLEDGMENTS

We thank Timothy Hey for his contributions at the initial stages of this project, Glenn Herrick for constructive criticism of the manuscript, and Jerri Cohenour for typing the manuscript.

This work was supported by Public Health Service grants GM23917 and GM07464 from the National Institutes of Health.

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