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Biochemistry

Identification of a second *Escherichia coli groE* gene whose product is necessary for bacteriophage morphogenesis

(*Escherichia coli groE* and *mop* mutants/isoelectric point variants)

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ABSTRACT **Previous work has uncovered the existence of an *Escherichia coli* locus, *groE*, that is essential for bacterial growth, λ phage and T4 phage head morphogenesis, and T5 phage tail assembly. Our genetic and biochemical analyses of λ *groE*$^+$ transducing phages and their deletion and point mutant derivatives show that the *groE* locus consists of two closely linked genes. One *groE* gene, *groEL*, has been shown to encode the synthesis of a 65,000 M_r polypeptide, whereas the second, *groES*, codes for the synthesis of a 15,000 M_r polypeptide. About half of the *groE*$^-$ bacterial isolates fall into the *groES* complementation group. *GroE* mutations in either gene cause similar phenotypes, with respect to λ phage head morphogenesis and bacterial growth at nonpermissive temperatures.**

The *Escherichia coli groE* locus codes for a major cellular protein that has been shown to be required for a wide variety of morphogenetic processes. *GroE*$^-$ mutants, although selected as bacteria that prevent productive infection by bacteriophage λ, are mutated in an essential bacterial function because many are temperature sensitive for growth, forming long filaments without septa at 42°C (1). Pleiotropic effects of these mutations include blocks on T4 head morphogenesis and T5 tail assembly (2, 3). Wild-type phage λ infecting *groE*$^-$ bacteria are blocked in head morphogenesis (4, 5) because only abnormal head structures are formed and none of the known proteolytic cleavages required for normal head assembly takes place (6, 7). This is consistent with the finding that the gene product of *groE* participates in an early step in λ prohead assembly, in which the *groE* product forms a complex with the gene product of *B* (7). The *groE*$^-$ mutations have been mapped at approximately 93 min on the *E. coli* map and are analogous to mutations called *mop* (8), *tabB* (9), and *hdh* (10).

The bacterial *groE* locus has been cloned by *in vitro* recombination into several λ vectors (7, 11, 12), which has allowed the identification of the gene product of *groE* as a polypeptide of 65,000 M_r. The purified polypeptide is found as a decatetramer sedimenting at 25 S, its 14 subunits arranged with 7-fold symmetry (13, 14). A weak ATPase activity is associated with the complex, but the normal function of this protein in uninfected bacteria is not known.

In this paper, we present a genetic and biochemical analysis of phage mutants from which we conclude that mutations in a second bacterial gene also cause the GroE phenotype. We have identified the product of this gene as a polypeptide of 15,000 M_r. We propose to rename the two *groE* genes *groEL* (which codes for the synthesis of the 65,000 M_r polypeptide) and *groES* (which codes for the synthesis of the 15,000 M_r polypeptide). According to this new designation, mutants originally named *groEA44* and *groEB764* (4) will be referred to as *groEL44* and *groEL764*, respectively.

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METHODS AND MATERIALS

Bacterial and Phage Strains. Most bacterial and phage strains used in these studies have been described (1, 2, 7, 11). The term *sup*$^+$ denotes nonsuppressing bacteria (formerly known as *sup*°, *sup*$^-$ or *su*$^-$). Phage H18a is an *i*$^\lambda$*c*I60 derivative of phage H18 (11). Phage H18 is an *i*^{21}*groE*$^+$*att*$^{\lambda+}$ transducing phage that was constructed *in vitro* by the insertion of the *Hin*dIII *E. coli* DNA fragment carrying the *groE*$^+$ genes into phage vector 540 (15). Phage 375 was constructed *in vitro* by the insertion of the *Eco*RI *E. coli* DNA fragment carrying the *groE*$^+$ genes into phage vector λ *gtB* (7, 16). Phage 378 is identical to phage 375 except that the orientation of the *Eco*RI *E. coli* DNA fragment has been reversed (Fig. 1). Media and bacterial and phage platings were as described (4, 7, 11).

Isolation of Phage-Deletion Derivatives. Deletion mutants of *groE*$^+$ transducing phages were isolated after EDTA treatment (17). Approximately 10^9 phage were treated with 2 mM EDTA for 1 hr at 42°C. Inactivation was stopped by the addition of $MgCl_2$ to 0.17 M. The surviving phage were plated on B178 *groE*$^+$ bacteria on Tryptone plates supplemented with 0.6 mM EDTA. Under these conditions, only phage which have undergone substantial deletion of DNA will form large plaques. This step is necessary because EDTA selection in liquid does not altogether eliminate phage with a full complement of DNA. Surviving phage were subsequently tested for growth on a *groES*$^-$ mutant (*groES30*) and on a *groEL*$^-$ mutant (*groEL140*).

Isolation of H18a*groELam1*. The level of mutants in phage H18a*groE*$^+$ was raised by growth on *mutD* bacteria (18) in the presence of 5 μg of thymidine per ml; this phage stock was adsorbed onto *groEL140supE* bacteria and plated on a 1:2 mixture of *groEL140supE* and *groEL140sup*$^+$ bacteria, respectively. After overnight growth at 37°C, small turbid plaques were further tested for growth on B178 *groE*$^+$ and *groEL140sup*$^+$ bacteria. Phage that made plaques on the former but not on the latter bacteria were tested for growth on *groEL140supE*. One mutant, H18a*groELam1*, that fulfilled these criteria for possessing an amber mutation in the *groEL* gene was found among approximately 30,000 phage progeny grown on *mutD* bacteria and tested.

Labeling Experiments. The bacterial strains, media, and procedures for labeling with either $^{35}SO_4^{-2}$ or [^{35}S]methionine have been described (11). The procedure for labeling phage-infected, UV-irradiated bacteria with a mixture of ^{3}H-labeled amino acids (^{3}H-amino acids) (20 μCi/ml; 1 Ci = 3.7×10^{10} becquerels) was identical to the conditions for labeling with [^{35}S]methionine, except that no unlabeled amino acids were added to the M9 medium. $^{35}SO_4^{-2}$, [^{35}S]methionine, and the mixture of the ^{3}H-amino acids were purchased from New England Nuclear (NET-250).

Phage DNA Preparation, Restriction Enzyme Digestion, Ligation, and Agarose Gel Electrophoresis. These techniques

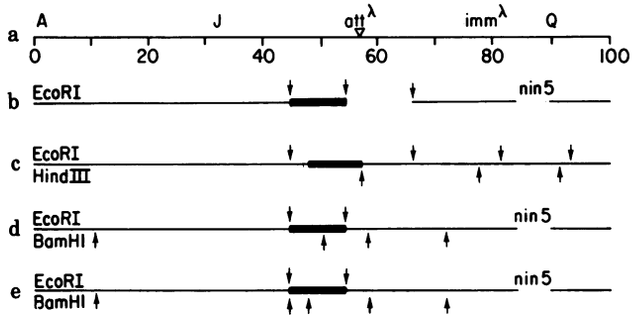

FIG. 1. Physical and genetic maps of phage λ vectors used in this work. The wild-type genome is divided into 100 units. Heavy lines indicate the position of the *groE*$^+$ bacterial DNA. Gaps indicate deleted DNA. Arrows mark positions at which the indicated restriction enzymes cleave. Maps: a, wild-type bacteriophage λ; b, W3; c, H18a; d, 375; and e, 378.

were described (7, 19). Likewise, NaDodSO$_4$/polyacrylamide gel electrophoresis was as described (11).

Two-Dimensional Gel Electrophoresis. The technique used was essentially that described by O'Farrell (20). The first dimension consisted of isoelectric focusing to equilibrium (6400 V/hr) in 1.6% (wt/vol) and 0.4% Ampholine mixtures (pH 5–7 and 3–10, respectively) in a 4% (wt/vol) polyacrylamide gel. The second dimension was run in NaDodSO$_4$/12.5% polyacrylamide gels.

Preparation of λ Proheads. The procedure for isolating λ proheads labeled with $^{35}SO_4^{-2}$ was as described by Hendrix and Casjens (6).

RESULTS

W3α Mutant. W3 is a λ*groE*$^+$ transducing phage that carries, in its insert of *E. coli* DNA, all genes necessary for growth on *groE*$^-$ hosts. We have reported the isolation of a mutant of W3, called W3α, which was no longer able to propagate on *groEL743* bacteria (11). When this mutant was tested on other *groE*$^-$ strains, it was found to divide the strains into two classes by its plating behavior (Table 1). Class I *groE*$^-$ bacterial mutants did not propagate W3α, whereas class II *groE*$^-$ bacterial mutants did. Because the α mutation affects the electrophoretic mobility of the 65,000 M_r protein (11), it was presumed to reside in the *groE* structural gene. We suggested two complementation models to explain the plating properties of W3α phage. The first model assumed that the 65,000 M_r protein is the only *groE* gene product and that the ability of W3α to plate on class II *groE*$^-$ bacterial mutants is due to intragenic complementation between the mutant subunits synthesized by W3α and the mutated host *groE* subunits. The fact that the purified form of the *groE* gene product is a decatetramer (13, 14) is consistent with such a model. The second model, intergenic complementation, was that there are at least two *groE* genes, and mutations in either one can cause the same bacterial phenotype. According to this model, W3α is a mutant in the *groEL* gene coding for the 65,000 M_r polypeptide and is able to grow on class II *groE*$^-$ bacterial mutants because class II mutants have a normal *groEL* gene but are mutated in a second *groE* gene, designated *groES*. Because both the temperature-sensitive phenotype for bacterial growth at 42°C and the abnormal λ prohead structures formed at 37°C are similar for *groE*$^-$ mutants in either class (Fig. 2*A*; unpublished data), these characteristics could not be used to distinguish between these models.

Isolation of a *groEL* Amber Mutant. To distinguish between the possibilities of intragenic and intergenic complementation, we searched for suppressible amber mutants in the *groEL* gene, which would be less likely to form functional hybrid complexes. One such mutant, called *groELaml*, was isolated after mutagenesis of H18a*groE*$^+$ transducing phage by growth on *mutD* bacteria. This mutant was selected on the basis of its ability to plate well on *groE*$^+$*sup*$^+$ and *groES30sup*$^+$ bacteria, but not on *groEL140sup*$^+$ bacteria (Table 1). When tested further on our other *groE*$^-$ strains, the growth pattern of phage H18a*groELaml* on *sup*$^+$ hosts was identical to that of W3α (Table 1). That is, it plated well on *groE*$^+$*sup*$^+$ and *groES*$^-$*sup*$^+$ bacteria, but did not grow on *groEL*$^-$*sup*$^+$ bacteria. That the *groELaml* mutation is indeed suppressible was shown by the ability of the phage to propagate on *groEL*$^-$*supE* or -*supF* bacteria (Table 1). This was verified by examining the proteins encoded by phage carrying the *groELaml* mutation. This was done by infecting UV-irradiated bacteria, labeling the proteins with radioactive precursors, and displaying them on NaDodSO$_4$/polyacrylamide gels. It was found that the 65,000 M_r protein band was absent in *sup*$^+$ infections (Fig. 2*B*, lane 3) but was present when *supE* bacteria were used as hosts (Fig. 2*C* lane 3). A potential amber fragment of 35,000 M_r can be seen upon infection of *sup*$^+$ bacteria by *groELaml* phage (Fig. 2*B*, lane 3). Because phage *groELaml* grows on *groES*$^-$*sup*$^+$ mutants, although it synthesizes only a fragment of gene product *groEL*, it seems unlikely that its ability to grow is due to intragenic complementation.

Isolation of Deletion Derivatives of H18a. To facilitate work with the various phage derivatives, we used the *groE*$^+$ transducing phage, H18a, which is *att*$^{\lambda+}$ (Fig. 1). Phage H18a was treated first with EDTA in liquid and then plated on EDTA-containing plates. Under these conditions, only phage that have suffered a significant loss of DNA will form plaques (17). Upon further testing, the H18a deletion derivatives fell into three groups. In all cases, the position and extent of the deletions found in each group have been analyzed genetically (see below) by DNA restriction mapping (Fig. 3) and by DNA heterodu-

Table 1. Plating properties of *groE*$^+$ transducing phage and their derivatives

Host *E. coli* strain	Phage strains*									
	λ	W3	W3α	H18a	Δ6† (group 1)	Δ3† (group 2)	Δ13† (group 3)	H18a *groELaml*	378	Δ101† (group 4)
B178 *groE*$^+$	+	+	+	+	+	+	+	+	+	+
groEL140sup$^+$	−	+	−	+	+	−	−	−	+	+
groEL140supE,F	−	+	−	+	+	−	−	+	+	+
groES30sup$^+$	−	+	+	+	+	−	+	+	+	−
groES30supF	−	+	+	+	+	−	+	+	+	−

* +, an efficiency of plating of 0.5–1.0; −, an efficiency of plating of $<10^{-3}$ at 37°C. The efficiency of plating on B178 *groE*$^+$ at 37°C is taken as 1.0.

† Phages Δ6, Δ3, and Δ13 are deletion derivatives of H18a, whereas phage Δ101 is a deletion derivative of 378.

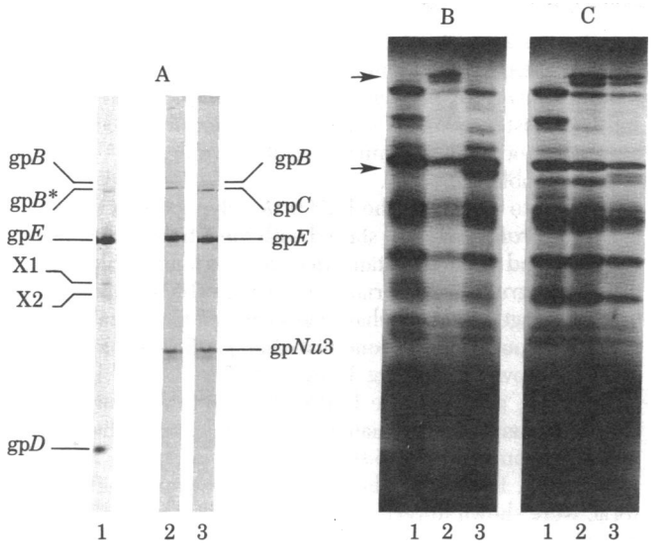

FIG. 2. Autoradiograms of $NaDodSO_4$/polyacrylamide slab gel electrophoresis. (*A*) λ proheads produced after the induction of λcI857 *Sam7* lysogens of B178 *groE*$^+$ (lane 1) *groES7* (lane 2) and *groEL44* (lane 3) bacteria. The proheads were labeled with $^{35}SO_4^{-2}$ and separated in glycerol gradients as described (6). Wild-type λ proheads are composed of X1 and X2 and gene products of *B*, *B**, *E*, and *D*, whereas λ proheads produced on *groE* hosts are composed of gene products of *C*, *E*, *Nu3*, and *B*. (*B*) UV-irradiated 159*uvrA*$^-$*sup*$^+$ bacteria infected with various phage and labeled with [^{35}S]methionine (20 μCi/ml) for 10 min at 37°C. Lanes: 1, λcI60; 2, H18a; and 3, H18a *groELam1*. The top arrow points to the 65,000 M_r gene product, which migrates as a doublet, and the bottom arrow points to the putative *groELam1*-coded amber fragment. (*C*) UV-irradiated *YmelsupF* bacteria infected with the same phages as above. The acrylamide was 12.5%.

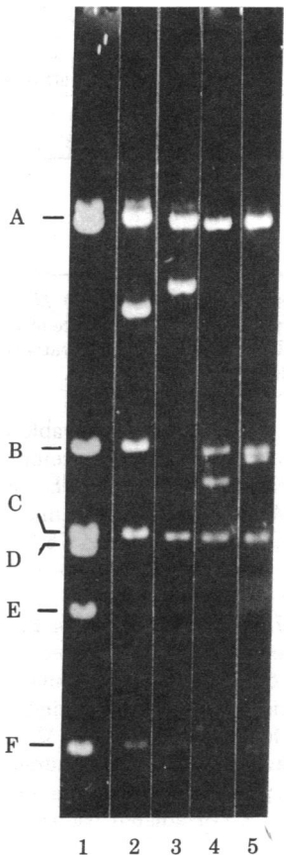

FIG. 3. *Eco*RI digests of various phage DNAs. Lane 1, λcI60. The fragments are named A, B, C, D, E, and F in order of decreasing size. Lane 2, H18a. This DNA has a new fragment generated by the bacterial DNA insertion, which replaces λ fragments D and E. Lane 3, H18aΔ6. This DNA is deleted from the *att*$^\lambda$ site to the right, fusing the new H18a fragment with the λ immunity-containing B fragment. Lanes 4 and 5, H18aΔ3 and H18aΔ13, respectively. These DNAs are deleted from the *att*$^\lambda$ site to the left ending in the bacterial DNA substitution, thus reducing the size of the new H18a fragment.

plexing experiments (unpublished results). Group 1 phage, exemplified by H18aΔ6, grow on all our *groE*$^-$ bacterial mutant strains. The majority of group 1 deletions originate at the attachment site and delete phage DNA sequences to the right (Fig. 3, lane 3). Group 2 phage, exemplified by H18aΔ3, do not propagate on any *groE*$^-$ bacterial strains tested. Group 2 deletions originate at the attachment site but proceed to the left, eliminating both phage DNA and bacterial DNA sequences, including the *groE* genes (Fig. 3, lane 4). Group 3 phage, exemplified by H18aΔ13, have deletions that also originate at the attachment site and proceed to the left but end in the middle of the *groE* DNA sequences (Fig. 3, lane 5). We came to this conclusion because H18aΔ13 phage grow well on all *groES*$^-$ bacterial mutants (on which W3α phage propagate) but do not grow on the *groEL*$^-$ bacterial mutants.

The correlation of growth of group 3 deletion phage and W3α is perfect (Table 1): among 21 *groE*$^-$ bacterial mutants tested, all those able to plate group 3 phage also plated W3α. Conversely, all the bacterial mutants unable to plate group 3 phage were also unable to plate W3α. Group 3 phage deletions account for only a few percent of all deletions found. This is not surprising, because they must terminate within the *groE* DNA region in order to eliminate one but not both of the *groE* activities. By restriction mapping the extent of the DNA deleted in representative group 2 and group 3 deletions, we have concluded that the two *groE* genes cannot be separated by more than 2,000 base pairs. We were unable to isolate any H18a deletion derivatives exhibiting the opposite spectrum of growth from that of group 3, that is, mutants that would grow on *groEL*$^-$ but not on *groES*$^-$ bacteria (group 4 deletion phages). Such deletions should only arise in phage H18a from a non-*int* gene-product-promoted recombination event originating to the left of or within the *groE* bacterial genes. However, deletions with such properties should be generated by the gene product of *int* if the orientation of the bacterial piece of DNA relative to *att*$^\lambda$ were reversed.

We constructed such a phage derivative as follows. Phage λ *groE*$^+$ vector 375 DNA (Fig. 1) was digested with *Eco*RI to release the two phage DNA arms and the *groE*$^+$-containing bacterial DNA fragment. The three DNA fragments were religated and packaged *in vitro*, as described (7). Ten plaques were picked at random, phage stocks were grown, and their DNA restriction patterns were analyzed after *Bam*HI digestion (21). Knowing that the *Eco*RI DNA fragment of *E. coli* carrying the *groE*$^+$ genes contains an asymmetric *Bam*HI restriction site, we anticipated that the *Bam*HI DNA restriction pattern of phage carrying the *groE*$^+$ genes in one orientation relative to the phage genes would be different from that of phage carrying the *groE*$^+$ insert in the opposite orientation. Of the 10 phages, 6 were shown by *Bam*HI digestion to have the insert in one orientation (e.g., 375), whereas the other 4 had the opposite orientation (e.g., 378). We showed that phage H18a and 375 have the bacterial DNA in the same orientation by DNA heteroduplexing. This was verified by the isolation of group 3 phage deletions from phage 375, with plating properties identical to H18aΔ13. Again, we were unable to isolate group 4 phage deletions. How-

Table 2. Proteins induced by λ *groE*$^+$ transducing phages and their derivatives

Protein, M_r	Relevant host genotype	Phage strains* λ	H18a	Δ6†	Δ3†	Δ13†	H18a *groELam1*	378	Δ101†
65,000	*sup*$^+$	−	+	+	−	−	−	+	+
65,000	*supF*	−	+	+	−	−	+	+	+
15,000	*sup*$^+$	−	+	+	−	+	+	+	−
15,000	*supF*	−	+	+	−	+	+	+	−

* +, Presence of the 65,000 M_r or 15,000 M_r protein species on NaDodSO$_4$/polyacrylamide gels; −, absence of the protein species.
† Phages Δ6, Δ3, and Δ13 are deletion derivatives of H18a, whereas phage Δ101 is a deletion derivative of 378.

ever, starting with phage 378, we were able to isolate group 4 phage deletions (i.e., those deletions exemplified by 378Δ101 and 378Δ102) which plate only on *groEL*$^-$ but not on *groES*$^-$ bacterial mutants. As expected, we were unable to isolate group 3 phage deletion mutants from phage 378 because the relative orientations of the *groE* bacterial genes and the *att*$^\lambda$ site had changed.

Identification of a Second *groE* Gene Product. To confirm the presence of the *groES* gene suggested by the genetic analysis, we examined the protein products made in UV-irradiated bacteria by the various λ*groE*$^+$ transducing phage. Previously, we were only able to identify the 65,000 M_r protein as the *groE* gene product because the W3α *groE*$^-$ mutant changed its electrophoretic mobility (11). Phage deletion mutants of all classes were used to infect UV-irradiated bacteria, and the proteins made were labeled with $^{35}SO_4^{-2}$, [^{35}S]methionine, or a mixture of ^{3}H-amino acids and analyzed on NaDodSO$_4$/polyacrylamide gels. Group 1 and 4 λ*groE* deletion mutants induced the synthesis of the 65,000 M_r protein (Fig. 4). As expected, λ*groE* deletion mutants in groups 2 and 3 did not induce the synthesis of the 65,000 M_r protein. However, a protein band corresponding to an approximate M_r of 15,000 was present in λ*groE*$^+$ and λ*groE* groups 1 and 3 deletion infections but was absent in λ *groE* groups 2 and 4 deletion infections (Fig. 4). The presence and absence of this protein species correlates exactly with the plating behavior of the various λ*groE* transducing derivatives and is consistent with its being the product of a second *groE* bacterial gene, which is mutated in all eleven *groES*$^-$ bacterial mutants (Table 2).

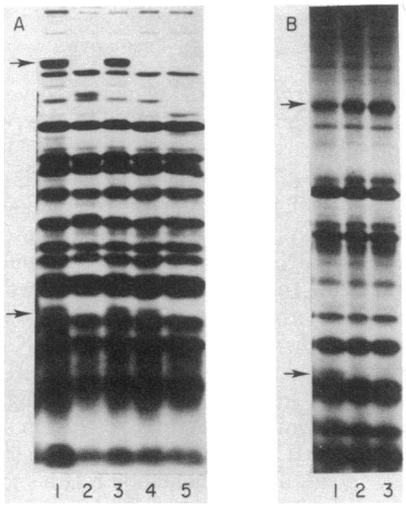

FIG. 4. Autoradiograms of NaDodSO$_4$/polyacrylamide slab gel electrophoresis. (*A*) UV-irradiated 159*uvrA*$^-$*sup*$^+$ bacteria infected with various phage and labeled with a mixture of ^{3}H-amino acids at 20 μCi/ml for 20 min at 37°C. Lanes: 1, H18a; 2, λcI60; 3, H18aΔ6; 4, H18aΔ13; and 5, H18aΔ3. The acrylamide concentration was a 10–25% linear gradient. (*B*) UV-irradiated 159 *uvrA*$^-$*sup*$^+$ bacteria infected with 378 (1), 378Δ101, (2) and 378Δ102 (3). The acrylamide was 12.5%. The arrows point to the positions of the 65,000 M_r and 15,000 M_r *groEL* and *groES* polypeptides.

In order to verify that the 15,000 M_r polypeptide is the product of the *groES* gene, we started with the λ transducing phage H18aΔ13 and isolated mutants that were no longer able to form plaques on *groES30* bacteria. None of the ≈100 independently isolated phage mutants behaved as expected for an amber nonsense mutation because none plated on *groES30supE* or *supF* bacteria. However, among 14 mutants tested, one, H18aΔ13 *groESKT10*, produced the 15,000 M_r protein at a normal rate after infection of UV-irradiated bacteria but altered the isoelectric point from pH 5.2 to 5.0 (Fig. 5*D*). Revertants of H18aΔ13 *groESKT10*, isolated by their ability to grow on *groES30* bacteria, were shown to recover simultaneously the wild-type isoelectric point (Fig. 5*E*). This result proves that the 15,000 M_r polypeptide is indeed the product of the *groES* gene.

DISCUSSION

It has been demonstrated that transducing phages carrying the *groE*$^+$ bacterial genes can be isolated as plaque formers on *groE*$^-$ mutant bacteria from a pool of transducing phages constructed *in vitro* so that they carry various segments of the *E. coli* chromosome (7, 11, 12). All these λ *groE*$^+$ transducing phages grow on all *groE*$^-$ bacterial mutants in our collection (11). However, the present studies indicate that our *groE*$^-$ bacterial mutants fall into two distinct complementation classes. *GroEL*$^-$ mutants, exemplified by *groEL743* and *groEL140*, are mutated in the gene encoding the 65,000 M_r polypeptide, whereas *groES*$^-$ mutants, exemplified by *groES7* and *groES30*, carry mutations in the gene encoding the 15,000 M_r polypeptide. The experimental basis for reaching this conclusion comprises the following data. (*i*) There exists a mutation in bacteriophage W3, called W3α, that affects both the ability to plate on *groEL*$^-$ bacteria and the electrophoretic mobility of the 65,000 M_r polypeptide. W3α grows normally on *groES*$^-$ bacterial mutants. (*ii*) An amber mutation affecting the 65,000 M_r polypeptide similarly affects growth on *groEL*$^-$ but not on *groES*$^-$ bacterial mutants. (*iii*) Deletion derivatives of the H18a, 375, and 378 λ*groE*$^+$ transducing phages exist that exhibit only one of the two *groE* complementing activities. Phages H18a and 375 have the bacterial DNA in the same orientation, with the relative order of the neighboring loci being (*J*$^+$*groES*$^+$*groEL*$^+$*att*$^\lambda$). Phage 378, however, has the opposite order (*J*$^+$*groEL*$^+$*groES*$^+$*att*$^\lambda$). Deletions of phages H18a and 375, starting at *att*$^\lambda$, produce *groES*$^+$*groEL*$^\Delta$ but not *groES*$^\Delta$*groEL*$^+$ derivatives, whereas deletions of phage 378 produce *groES*$^\Delta$*groEL*$^+$ derivatives but not *groES*$^+$*groEL*$^\Delta$ mutants. The existence of such deletions eliminates the possibility that the two observed *groE* complementation groups represent intragenic rather than intergenic complementation. (*iv*) There exists a perfect correlation between the presence of the 15,000 M_r polypeptide and *groES*$^+$ complementation activity. Similarly, the presence of the 65,000 M_r polypeptide is always associated with *groEL*$^+$ complementation activity. In spite of our efforts, we have been unable thus far to isolate amber mutations in the *groES* gene to further substantiate this conclusion. However, we have been able to isolate a transducing phage with a missense mutation of the *groES* gene causing a shift in the isoelectric point of the 15,000 M_r polypeptide from pH 5.2 to 5.0 (Fig. 5). Revertants of this mutant phage, isolated on the basis

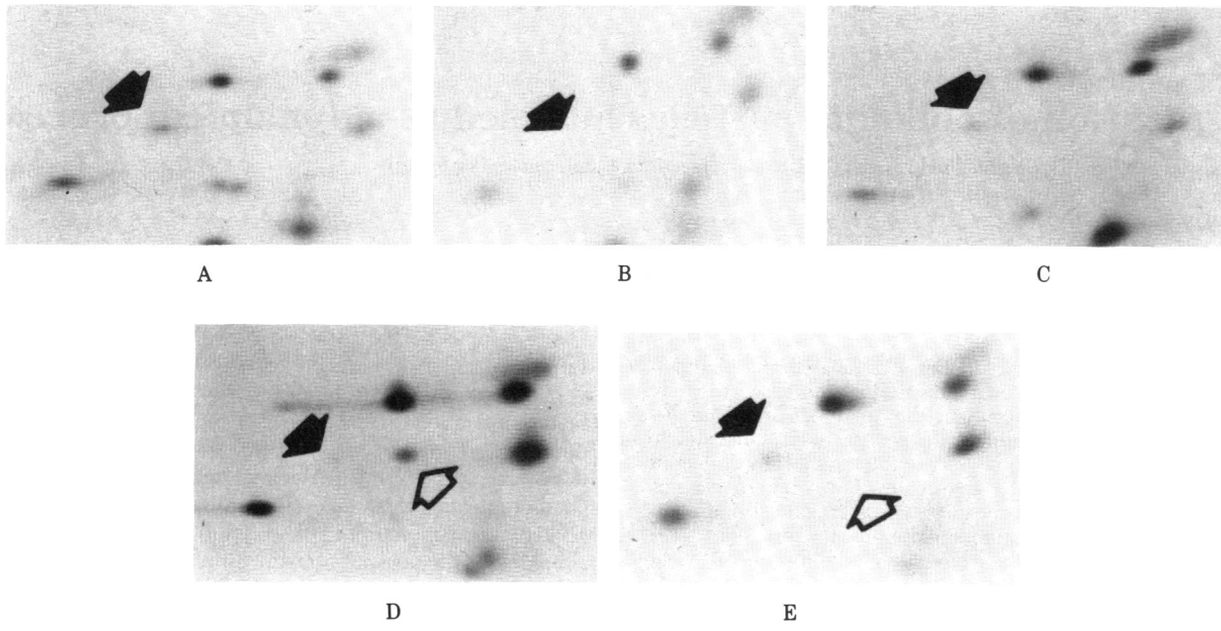

FIG. 5. Two-dimensional gel electrophoresis of infections of UV-irradiated 159*uvrA*$^-$*sup*$^+$ bacteria with H18a (*A*), H18aΔ3 (*B*), H18aΔ13 (*C*), H18aΔ13 *groESKT10* (*D*), and H18aΔ13 *groESKT10* revertant 1 (*E*). Labeling was as described for Fig. 2*B*. Only the regions of the gel corresponding to a 4.75–5.75 pH gradient (in the horizontal dimension) and 12,000–17,000 M_r (in the vertical dimension) are shown. The solid arrows point to the position of the wild-type 15,000 M_r polypeptide. Open arrows point to the position of the H18aΔ13 *groESKT10*-coded 15,000 M_r polypeptide.

of their ability to form plaques on *groES*$^-$ hosts, make a 15,000 M_r protein with the wild-type isoelectric point.

We have been unable to detect any differences in the GroE phenotypes exhibited by mutants in the two *groE* genes. Mutations in either gene seem to affect λ phage head morphogenesis at a very early step. The proheads isolated from λ phage infections of *groEL*$^-$ or *groES*$^-$ bacterial mutants show the same molecular composition (i.e., gene products of *E*, *Nu3*, and *C*, and a small amount of unprocessed product *B*) (Fig. 2*A*). Furthermore, there are *groE*$^-$ mutants in either gene that are temperature sensitive for bacterial growth at 42°C. From these results it appears that the 65,000 M_r and 15,000 M_r gene products either act at the same levels in λ phage and bacterial morphogenesis or act at separate steps of the same pathways. At this stage we cannot distinguish between these two possibilities. The position of *groEL* product action in λ head morphogenesis has been established (7), yet purified preparations of the native decatetramers do not contain detectable amounts of the 15,000 M_r polypeptide, suggesting that the two *groE* proteins do not form a tight enough complex to copurify. In preliminary experiments, however, we have shown that mutations in the *groEL* gene can partially suppress mutations in the *groES* gene, suggesting that the proteins do indeed interact *in vivo* (unpublished results). Elucidating the mode of action of the two *groE* bacterial gene products in bacteriophage λ, T4, and T5 morphogenesis and in *E. coli* growth will contribute significantly to our understanding of the assembly mechanism of complex macromolecular structures.

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