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Identification of a host protein necessary for bacteriophage morphogenesis (the *groE* gene product)

(transducing bacteriophage λ/sodium dodecyl sulfate gel electrophoresis/*in vitro* recombination)

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ABSTRACT **Mutations in the *groE* gene of *Escherichia coli*, which block the correct assembly of the phage λ head, have been previously described. Many *groE* mutations exert pleiotropic effects, such as inability to propagate phages T4 and T5 and inability to form colonies at 43°. With the help of the *Eco*RI and *Hin*dIII restriction enzymes and the appropriate phage vectors, we have constructed two λ transducing phages, called W3 and H18, that carry the *groE*$^+$ bacterial gene. Upon lysogenization by phage H18 the *groE* bacterial mutants recover their *gro*$^+$ phenotype for both phage growth and the ability to form colonies at 43°. We have identified the *groE*$^+$ bacterial gene product as a protein of 65,000 molecular weight. Mutants of the W3 transducing phage that were selected on the basis of their ability to propagate on some *groE* mutant hosts induce the synthesis of a *groE* protein with altered electrophoretic mobility.**

The morphogenesis of the bacteriophage λ head is a complex process that requires the products of at least ten phage-coded genes and one, *groE*, of the bacterial host (for review see refs. 1 and 2). Bacterial mutants in the *groE* gene have been isolated and found to block λ head morphogenesis (3, 4). When wild-type λ phage infects *groE* mutant bacteria, abnormal head structures are formed and the morphogenetic protein cleavages that are associated with proper head assembly do not occur (4, 5). Phage mutants, called λε, which can overcome the block exerted by the *groE* mutations, can be isolated and have been shown to map in the coat protein gene, *E*, or in some instances, gene *B* (3, 4). The gene *B* protein acts early in the head assembly pathway, before DNA packaging (1). In addition to blocking λ head morphogenesis, some *groE* mutations interfere with T4 head assembly at the level of action of the gene *31* product, as judged both by the phenotype of infection and the isolation of phage mutants in gene *31* that can bypass the bacterial block (6–8). Gene *31* is somehow required for proper T4 coat protein assembly very early in head morphogenesis, before the cleavage of any of the head proteins (9). In the case of bacteriophage T5 it has been found, surprisingly, that the effect of the *groE* mutation is primarily exerted at the level of tail assembly but not at that of the head (10). Again, a morphogenetic protein cleavage, in this case associated with tail assembly, does not occur in T5 infections of *groE* mutant bacteria. The groE mutations have been mapped at approximately 98 min on the revised genetic map of *Escherichia coli* (11, 12) and are thought to be analogous to *mop* (7), *tabB* (8), and *hdD* (13). In addition to blocking phage morphogenesis, most *groE* mutations render the bacteria temperature sensitive for growth at 43° (11, 12). Mapping experiments, as well as reversion studies, suggest that both the *groE* phenotype and bacterial temperature sensitivity are due to the same mutation. Thus, the *groE* gene product is indispensable for both phage and bacterial growth. In this paper, we report on the isolation of λ transducing phages carrying a functional *groE*$^+$ bacterial gene, the identification of the *groE* protein on sodium dodecyl sulfate/polyacrylamide gels, and the isolation and characterization of mutants in the *groE* gene carried on the transducing phage.

METHODS AND MATERIALS

Bacterial and Phage Strains. Most bacterial and phage strains used in these studies have been described previously (4, 12). *E. coli* K-12 159 *uvrA*$^-$ (λ*ind*$^-$) was used as the host in the UV-irradiation experiments. A pool of phage no. 540 (= λ∇*srI*λ1-2*shind*IIIλ3$^+$*att*$^+$*imm*^{21}*nin*R5*shind*IIIλ6^0) (14) and a pool of phage no. 569 (= λ∇*srI*λ1-2*srI*4^0*srI*5^0*cI*857 *gam*$^-$*am*210*nin*R5) carrying various restriction fragments of *E. coli* DNA were kindly provided by Ken Murray. The relevant genetic compositions of these phages are shown in Fig. 1.

Media and Bacterial and Phage Platings. These were as previously described (4, 12). Low-sulfur M9, for labeling with $^{35}SO_4^{2-}$, contains per liter: 7 g of Na_2HPO_4, 3 g of KH_2PO_4, 0.5 g of NaCl, 1 g of NH_4Cl, 11 mg of $CaCl_2$, 7 mg of $MgSO_4 \cdot 7H_2O$, 1 mg of thiamine·HCl, 20–50 mg of each amino acid except methionine and cysteine, and 4 g of maltose. High-sulfur M9, for labeling with [^{35}S]methionine, is low-sulfur M9 supplemented with 0.25 g of $MgSO_4 \cdot 7H_2O$.

Isolation of the W3α and W3β Phage. Phage W3, a *groE*$^+$ transducing phage isolated in the course of this work (see *Results*) was mutagenized either by hydroxylamine (15) or by means of single-cycle growth on K-12 *gro*$^+$ bacteria in the presence of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at 10 μg/ml. The mutagenized and segregated phage was adsorbed onto *groE1 sup*II bacteria and plated on a mixed bacterial indicator lawn consisting of a 1:1 mixture of *groE1 sup*II and *groE743 sup*^{0} bacteria (*sup*^{0} bacteria are not suppressing). After overnight incubation at 37°, plaques that appeared to be both small and turbid were further tested, by means of a toothpick, onto *gro*$^+$ *sup*^{0} and *groE743 sup*^{0} bacteria. Plaques that grew on *gro*$^+$ *sup*^{0} bacteria but failed to grow on *groE743 sup*^{0} were further analyzed for ability to grow on *groE1 sup*II bacteria. Among the 5000 mutagenized plaques tested, two mutants, called W3α and W3β, were found, which grew on both *gro*$^+$ *sup*^{0} and *groE1 sup*II bacteria but not on *groE743 sup*^{0} bacteria.

Construction of H18*imm*^{λ} Derivative Phage. Phage H18, an *imm*^{21}*groE*$^+$ transducing phage isolated in the course of this work (see *Results*), was crossed with phage λ*imm*^{λ}*cI*857 at a multiplicity of infection of 5 for each parent. Recombinant phage (called H18*imm*^{λ}) that carry both the *groE*$^+$-containing bacterial DNA segment and the *imm*^{λ} region were selected by plating on *groE743*(λ*imm*^{21}) bacteria.

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Abbreviation: M_r, molecular weight.

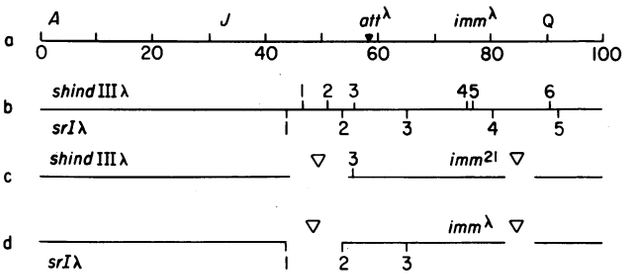

FIG. 1. Genetic structure of the phage λ vectors used in this work (after ref. 14). (*a*) Genetic map of wild-type bacteriophage λ DNA. The genome is divided into 100 units. (*b*) The positions of the *Hin*dIII restriction sites on wild-type λ DNA are shown on top of the line; those for *Eco*RI are below the line. (*c*) Phage vector 540. All the *Hin*dIII-sensitive sites have been eliminated, either by deletion or DNA substitution, except site 3. The bacterial DNA segment carrying the *groE* region is inserted at site 3 (see Fig. 2*C*). Thus, the resulting *groE*$^+$ transducing phage H18 (see text) possesses a normal *att*$^\lambda$ site. (*d*) Phage vector 569. This vector has two *Eco*RI-sensitive sites, site 3 and a hybrid site called 1/2. Site 1/2 is made up of the left half of site 1 and the right half of site 2 and is created by the removal of the phage DNA segment between these two sites after *Eco*RI digestion. The bacterial DNA segment carrying the *groE* region replaces the phage DNA segment between sites 1/2 and 3 (see Fig. 2*D*). Thus, the resulting *groE*$^+$ transducing phage W3 (see text) lacks the *att*$^\lambda$ region.

Labeling Experiments. Strain 159 *uvrA*$^-$(λ*ind*$^-$) was grown at 37° in low-sulfur M9 to 1.5×10^8 cells per ml. The culture was washed, concentrated 15-fold in 10 mM $MgCl_2$, UV irradiated (incident flux of approximately 15,000 ergs/mm^2 at 254 nm), and then infected with λ phage at a multiplicity of 5. After 15-min. adsorption at room temperature, the culture was diluted 15-fold into prewarmed low-sulfur M9 medium supplemented with 250 μCi of carrier-free $^{35}SO_4^{2-}$ (New England Nuclear) and shaken at 37° for 1 hr. The culture was centrifuged and the cell pellet was resuspended in 1/20 volume of electrophoresis sample buffer (15). For labeling with [^{35}S]-methionine, the same procedure was followed except that after UV irradiation the culture was resuspended in high-sulfur M9, grown at 37° for 30 min, and then pulsed for 7–8 min with 50 μCi of carrier-free [^{35}S]methionine (New England Nuclear). Incorporation was terminated with the addition of 10 volumes of −20° chilled acetone followed by centrifugation and resuspension in electrophoresis sample buffer. In chase experiments, unlabeled methionine was added at 50 μg/ml.

Electrophoresis. The procedures for preparing the samples and for discontinuous sodium dodecyl sulfate/polyacrylamide slab gel electrophoresis were described by Russel (16).

RESULTS

Isolation of *groE* Transducing Phages. Transducing phages carrying the *groE*$^+$ bacterial gene were isolated as plaque formers on *groE* mutant bacteria by plating a pool of transducing phages specifically constructed *in vitro* so that they carry various segments of the bacterial chromosome (17). One group of *groE*$^+$ transducing phages, exemplified by phage H18, (= 540*groE*$^+$) was constructed with the help of the *Hin*dIII restriction enzyme, starting from phage 540 (14) and *E. coli* *gro*$^+$ DNA. The frequency of occurrence of such *groE*$^+$ transducing phages was 1×10^{-4}. Another group, exemplified by phage W3 (= 569 *groE*$^+$), was constructed with the aid of the *Eco*RI restriction enzyme, starting from phage 569 and *E. coli* *gro*$^+$ DNA, at a frequency of 3×10^{-4}. The frequency of occurrence of both H18 and W3 was at least 1000-fold higher than that expected for λε mutants, which are point mutants that map in the *E* or *B* phage genes and have been previously shown to overcome the *groE* bacterial block (4). The rationale behind the selection of the *groE*$^+$ transducing phage was that such transducing phages will express the wild-type *groE*$^+$ product and thus help promote its proper head morphogenesis on *groE* mutant bacteria.

Table 2. Properties of H18 lysogens

Lysogen	Efficiency of plating* λ*imm*$^\lambda$*cI*	Efficiency of plating* T4D	Bacterial survival at 43°†
gro$^+$(540)	1.0	1.0	0.9
gro$^+$(H18)	1.0	1.0	0.9
*groE*A44(540)	$<10^{-7}$	2×10^{-6}	3×10^{-7}
*groE*A44(H18)	1.0	1.0	0.85

* The number of plaques produced on a bacterial host at 37° relative to *gro*$^+$ *E. coli* K-12.

† The number of colonies formed at 43° relative to the number of colonies formed at 30°.

Properties of H18 and W3. Upon testing, both H18 and W3 phages proved capable of propagating on all *groE* mutant host strains tested (Table 1). This result suggests that both phages, although constructed with different restriction enzymes, carry a bacterial segment that covers all the known *groE* mutations. It furthermore suggests that all the *groE* mutations in our collection are recessive to the wild-type allele. Upon lysogenization of *gro*E mutant bacteria by phage H18, which carries an intact *att*$^\lambda$ site, the resulting lysogens (20 out of 20 examined) become simultaneously permissive (*gro*$^+$) for lambdoid phages and temperature resistant for growth at 43° (Table 2). Both properties are compatible with H18 being a *groE*$^+$ transducing phage and not a λε mutant. The fact that the lysogens can express the *groE* function suggests that the *groE*$^+$ gene on the H18 phage chromosome is not subject to phage repressor control, i.e., it probably possesses its own promoter. Phage W3, which lacks the *att*$^\lambda$ region and hence can only lysogenize by virtue of its bacterial segment, also renders the *groE* host able to propagate heteroimmune λ phages and T4 (data not shown).

Physical Characterization of H18 and W3. We have characterized the bacterial DNA fragment inserted in phages H18 and W3 by a variety of methods including (*i*) density gradient centrifugation, (*ii*) cleavage of phage DNA with appropriate restriction enzymes and separation of the DNA fragments on agarose gels, and (*iii*) denaturation of the transducing phage DNA and reannealing with the parental phage DNA. Heteroduplex analysis has yielded estimates of ap-

Table 1. Plating properties* of H18 and W3 phage

Host *E. coli* K-12 genotype	Phage strain 540	H18	569	W3	W3α	W3β
gro$^+$	1.0	1.0	1.0	1.0	1.0	1.0
groE1, 3, 7, 97, 563	$<10^{-7}$	1.0	$<10^{-7}$	1.0	1.0	1.0
groE44, 136, 140, 639, 673, 743	$<10^{-7}$	1.0	$<10^{-7}$	1.0	$10^{-2} - 10^{-3}$	$10^{-2} - 10^{-3}$

* As judged by the number of plaques produced by a phage strain on a given bacterial host at 37° relative to the number on the *gro*$^+$ host.

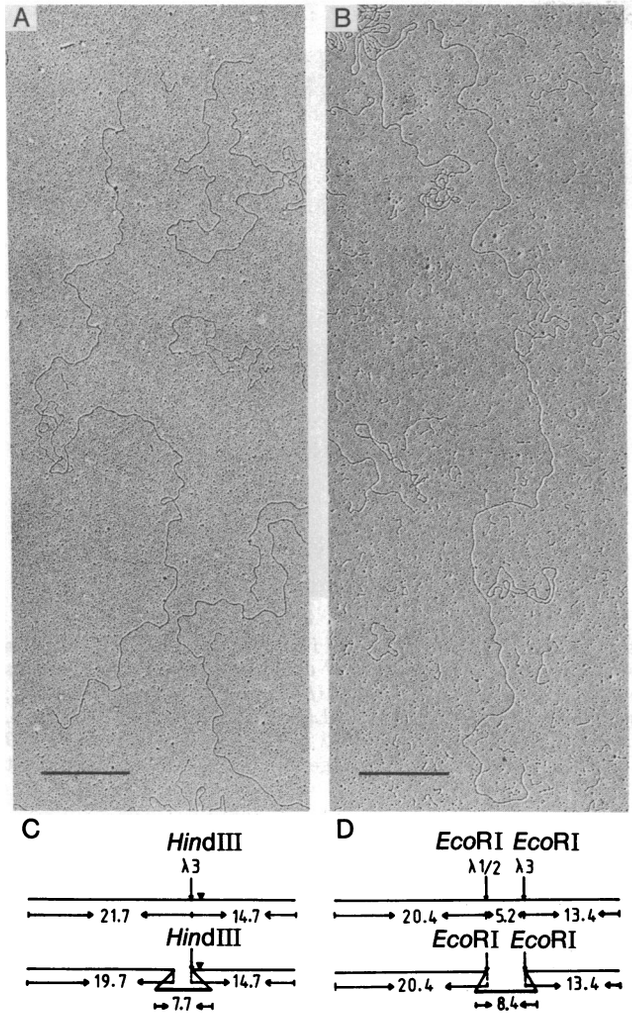

FIG. 2. Electron micrographs of λ heteroduplex DNA molecules. Heteroduplexes were prepared and mounted for electron microscopy as described by Davis *et al.* (18). After spreading, the molecules were picked up with parlodion-coated grids, shadowed with platinum, and observed in a Zeiss EM 10. Double-stranded circular phage PM2 DNA and single-stranded circular phage fd DNA were added to the spreading solution as internal standards. (*A*) Heteroduplex formed between H18 and its parent phage 540; (*B*) heteroduplex formed between W3 and its parent phage 569. The bar represents 1 μm. (*C* and *D*) Measured lengths, in kilobases, of H18/540 and W3/569 heteroduplexes, respectively. Vertical arrows mark cleavage sites for the restriction endonucleases indicated. The segment represented by the heavy line is bacterial DNA. ▼ Indicates att^{λ}.

proximately 7700 and 8400 base pairs for the bacterial DNA inserted in H18 and W3 phages, respectively. Fig. 2 shows the heteroduplex molecules formed by (*A*) annealing the H18 transducing phage to its 540 parent and (*B*) annealing the W3 transducing phage to its 569 parent. Fig. 2 *C* and *D* shows measurement and interpretation of *A* and *B*, respectively. The loss of a piece of phage-specific DNA upon formation of the H18 phage explains the double loop in the heteroduplex with the parent phage and the loss of a *Hin*dIII restriction site. A possible mechanism for this illegitimate recombination has been suggested (19).

Characterization of the *groE* Bacterial Product. The fact that a *groE* mutant strain, when lysogenic for H18 phage, recovers its gro^+ phenotype suggests that the $groE^+$ gene carried by the H18 prophage is expressed in the lysogenic state. We took advantage of this observation to characterize the *groE* product. *E. coli* K-12 159 $uvrA^-$ sup^0 cells lysogenic for λind^- (whose repressor is not inactivated by UV irradiation) were UV irradiated to reduce host protein synthesis and were subsequently infected with various λimm^{λ} derivative phage. Fig. 3*A* shows that infection by H18imm^{λ} or W3imm^{λ} specifically causes the synthesis of a new protein species with a molecular weight (M_r) of 65,000. The other protein species that are also synthesized occasionally under these conditions are probably phage-specific proteins, because they are made by wild-type λ or a H18imm^{λ} derivative phage carrying a heterologous piece of bacterial DNA that does not include the *groE* region. Hence, the only bacterial-specific protein made by the transducing phages is the 65,000-M_r protein.

In order to test whether the 65,000-M_r protein represents the *groE* gene product, we searched for nonsense (amber) mutants in the *groE* gene carried by phage W3. Phage W3 was mutagenized by either hydroxylamine or nitrosoguanidine and screened for derivatives that could form plaques on *groE1 sup*II but not on *groE743* sup^0 bacteria. Two such mutants, called W3α and W3β, that fulfilled the criteria of the selection procedure were isolated. Upon further characterization, however, they proved to possess no amber mutation in the *groE* gene. Rather, they appeared to be host-specific mutants in the *groE* gene, e.g., they propagate on both *groE1 sup*II and *groE1* sup^0 bacteria, but not on *groE743 sup*II or *groE743* sup^0 bacteria. Table 1 summarizes the plating properties of W3α and W3β phage. Approximately half of the *groE* strains tested propagated the W3α and W3β phages and the rest did not. No real amber mutations were found among approximately 5000 mutagenized plaques of W3 phage screened. We do not understand the reason for this failure.

In spite of the fact that the α and β mutations do not appear to be of the nonsense variety, sodium dodecyl sulfate/polyacrylamide electrophoresis showed that they both affect the electrophoretic mobility of the 65,000 M_r protein, altering its apparent molecular weight to 67,000 and 70,000, respectively (Fig. 3 *B* and C). This altered electrophoretic mobility is not host specific, e.g., it is the same whether the phage is grown on 159$uvrA^-$(gro^+), on *groE1 sup*II (permissive *groE* mutation for W3α and W3β phage), or on *groE743* sup^0 (nonpermissive for W3α and W3β phage) (results not shown).

When W3α and W3β phage were plated on the nonpermissive *groE743* bacteria, "revertant" plaques appeared at a frequency of 10^{-2}–10^{-3} (Table 1). Two such revertants of W3α and W3β were tested and shown to induce the synthesis of the 65,000 M_r protein (Fig. 3*C*). Because the only selection exerted in the isolation of these "revertants" was simply their ability to propagate on *groE743* bacteria, the 65,000 M_r protein species most likely represents the product of the *groE* gene.

Various control experiments suggest that the altered electrophoretic mobility of the 65,000 M_r protein induced by the W3β mutation is not an artifact of experimental procedure. Experiments such as (*a*) mixing the protein extracts of separate infections by W3 and W3β phage (results not shown) or (*b*) mixedly infecting bacteria with both W3 and W3β (Fig. 3*D*) proved that the altered mobility induced by the W3β mutation is always expressed, suggesting that it is indeed a direct consequence of the presence of the W3β mutation.

DISCUSSION

Two λ transducing phages, H18 and W3, have been isolated and characterized which carry the $groE^+$ bacterial gene as judged by their ability to propagate on all *groE* mutant strains in our collection. Their frequency of occurrence (approximately

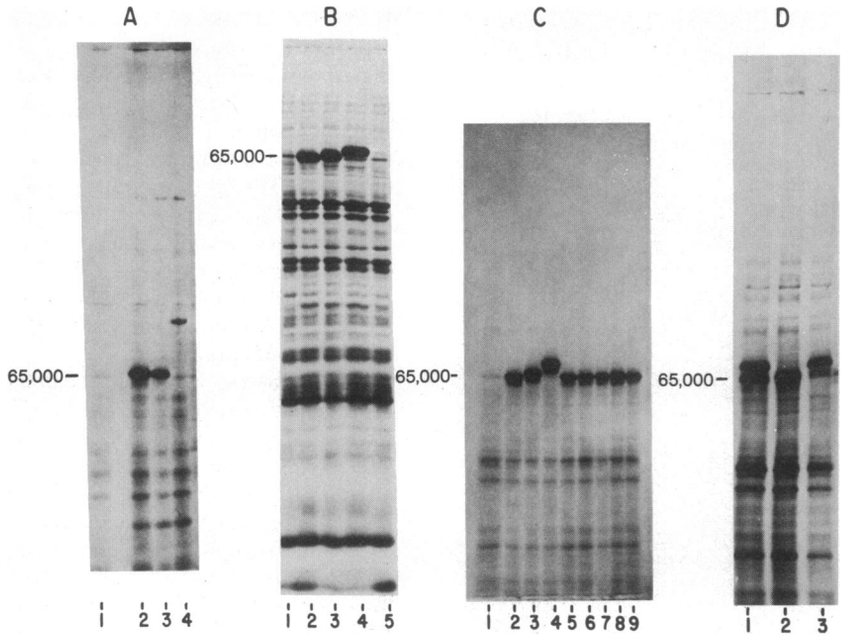

FIG. 3. Autoradiograms of sodium dodecyl sulfate/polyacrylamide slab gel electrophoresis of UV-irradiated 159 *uvrA*$^-$(*imm*$^\lambda$*cI*$^+$*ind*$^-$) bacteria infected with various phages. (*A*) Lane 1, no phage; 2, W3 phage; 3, H18*imm*$^\lambda$ phage; and 4, a phage 540 *imm*$^\lambda$ derivative carrying a heterologous piece of bacterial DNA that does not include the *groE*$^+$ gene. The percent acrylamide in the slab gel was 7.5. (*B*) Lane 1, no phage; 2, W3 phage; 3, W3α phage; 4, W3β phage; and 5, 569 parent phage. The percent acrylamide in the slab gel was 12.5. (*C*) Lane 1, no phage; 2, W3; 3, W3α; 4, W3β; 5 and 6, two W3α → W3 "revertants" isolated on *groE743* bacteria; and 7, 8, and 9, three W3β → W3 "revertants" isolated on *groE743* bacteria. The percent acrylamide in the slab gel was 7.5. (*D*) Lane 1, both W3 and W3β phages; 2, W3 phage; and 3, W3β phage. The percent acrylamide in the slab gel was 7.5.

10^{-4}) is substantially higher than that expected of $\lambda\epsilon$ mutants (10^{-7}–10^{-9}), which can also bypass the *groE* bacterial block (3, 4). Upon lysogenization by both phages the *groE* mutant strains lose both their ability to block λ head morphogenesis and their temperature sensitivity. These properties prove that H18 and W3 are indeed *groE* transducing phages and not $\lambda\epsilon$ mutants.

Infection of UV-irradiated bacteria by H18 of W3 phage has allowed the identification of a 65,000-M_r protein species. We believe this protein to be the product of the *groE* gene. The evidence is mostly based on the isolation of the W3α and W3β phage (derived from phage W3) which, having lost the ability to plate on some *groE* mutant strains, simultaneously induce the synthesis of protein species with electrophoretic mobility somewhat higher than 65,000. This difference in electrophoretic mobility is not dependent on whether the phages infect permissive *groE* bacteria (such as *groE1 sup*II or *sup*^{0}) or nonpermissive *groE* bacteria (such as *groE743 sup*II or *sup*^{0}). W3α and W3β phage, simultaneously with reversion to plaque-forming ability on *groE743*, recover the ability to synthesize the 65,000-M_r protein. This strongly suggests that this protein is the product of the bacterial *groE* gene. The high frequency of such "revertants," 10^{-2}–10^{-3}, suggests that they are not true revertants, but rather, they represent recombinants between the altered *groE* allele carried by the W3α and W3β phage and the *groE*$^+$ or *groE* mutant allele of the host on which they were grown.

It is not yet clear how the W3α and W3β mutations alter the electrophoretic mobility of the *groE* protein. W3α and W3β could simply be missense mutations in the *groE* gene that alter the electrophoretic mobility on sodium dodecyl sulfate gels. Alternatively, they could cause synthesis of longer polypeptide chains, although the mechanism by which such proteins may have arisen remains obscure. A possibility to be considered is that the *groE* protein itself is either proteolytically cleaved or otherwise modified and that the W3α and W3β mutations interfere with the completion of this processing. If this were the case, the processing must be very rapid, because we were unable to detect any precursor species of the *groE*$^+$ gene product during a 140-sec pulse with [^{35}S]methionine (unpublished results).

If the 65,000-M_r protein is the only bacterial *groE* product, the fact that the W3α- and W3β-induced *groE* products can function successfully on some *groE* hosts but not on others suggests the possibility of intragenic complementation between the W3α- or W3β-altered *groE* protein. This, in turn, would suggest that the active *groE* product is made up of two or more subunits. On the other hand, a possibility which we have not excluded is that the *groE*$^+$ activity is made up of more than one polypeptide chain, say A (the 65,000-M_r product) and B. Because our W3 and H18 transducing phages propagate on all our *groE* mutants tested, they must code for both polypeptide chains. The W3α and W3β mutations could be mutations affecting only the 65,000-M_r protein and would thus fail to propagate on all *groE* bacterial mutants defective in this protein but would propagate (e.g., complement) on all *groE* bacterial mutants defective in the B polypeptide. If the multiple polypeptide hypothesis is correct, the failure to detect the synthesis of the other polypeptide chains could be due (*a*) to instability, (*b*) to limited synthesis, (*c*) to containing no methionine or cysteine, or (*d*) to masking by a host protein on the sodium dodecyl sulfate gels. No complementation has yet been done to determine if indeed our *groE* isolates fall into more than one complementation group.

Hendrix and Tsui (20) have been successful in obtaining amber mutations in the *groE* gene carried by a similarly constructed transducing phage. Their conclusion that the 65,000-M_r protein is the product of the *groE* bacterial gene is in agreement with ours.

The role that the *groE* product plays in phage λ morphogenesis is not known. Now that it has been identified it should

be possible to look for its presence in λ pro-head structures or in other complexes with λ morphogenetic proteins and, eventually, to purify it.

We gratefully appreciate having received from Ken Murray large quantities of phage with recombinant DNA of λ and *E. coli*. Doris Bitterli prepared and measured the heteroduplex molecules, Ruth Bisig provided excellent technical assistance, and Thomas Hohn, Sherwood Casjens, and Harvey Eisen provided constructive criticism in the preparation of the manuscript. We thank Roger Hendrix for communicating his results prior to publication. Financial support was provided by a grant to T. Hohn from Schweizer National-Fonds, by Grant 3.519.75 from the Fonds National Suisse de la Recherche Scientifique, and by National Institutes of Health Grant GM23917-01.

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