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Escherichia coli grpE Gene Codes for Heat Shock Protein B25.3, Essential for Both λ DNA Replication at All Temperatures and Host Growth at High Temperature

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We have identified the *grpE* gene product as the B25.3 heat shock protein of *Escherichia coli* on the following evidence: (i) a protein similar in size and isoelectric point to B25.3 was induced after infection of UV-irradiated bacteria by λ *grpE*$^+$ transducing phage, (ii) mutant phage λ *grpE40*, isolated by its inability to propagate on *grpE280* bacteria, failed to induce the synthesis of the B25.3 protein, and (iii) λ *grpE*$^+$ revertants, derived from phage *grpE40* as able to propagate on *grpE280* bacteria, simultaneously recovered the ability to induce synthesis of the B25.3 protein. In addition, we show that *E. coli* bacteria carrying the *grpE280* mutation are temperature sensitive for bacterial growth at 43.5°C. Through transductional analysis and temperature reversion experiments, it was demonstrated that the *grpE280* mutation is responsible for both the inability of λ to replicate at any temperature tested and the lack of colony formation at high temperature. At the nonpermissive temperature the rates of synthesis of DNA and RNA were reduced in *grpE280* bacteria.

The identification of *Escherichia coli* proteins necessary for bacteriophage λ DNA replication has been facilitated by the isolation of bacterial mutants (originally termed *groP* or *grp*) that fail to support phage growth (6, 7). The combined efforts of many different laboratories have demonstrated that the host *dnaB*, *dnaK*, *dnaJ*, *grpD*, and *grpE* gene products are absolutely necessary for λ DNA replication (6–8, 10, 14, 21, 23). The block to λ DNA replication can be overcome by phage mutations (originally termed π [10] or *reg* [21]) that map in the *P* gene, suggesting that the five host proteins and the phage-coded P protein functionally interact. In support of this, it has been demonstrated that the purified P protein of λ physically interacts with both the DnaB (29) and DnaK bacterial proteins (31). Recently it has been shown that the DnaK and DnaJ proteins belong to the heat shock class of proteins (2, 12). The rate of synthesis of the heat shock proteins is positively regulated by the *htpR* (*rpoH*) gene product and is coordinately and transiently increased after a temperature shift (13, 19). In this paper we demonstrate through genetic and biochemical analyses that the GrpE protein is identical to the B25.3 heat shock protein of *E. coli* originally identified by Neidhardt and colleagues (19). The *grpE* gene, which encodes a 24,000-M_r protein (20), was discovered because a mutation in it, *grpE280*, blocked bacteriophage λ DNA replication at all temperatures (21). Thus, at least three of the host proteins, DnaJ, DnaK, and GrpE, known to participate in λ DNA replication are heat shock proteins. In addition we demonstrate that the GrpE protein is essential for host RNA and DNA syntheses at high temperature.

MATERIALS AND METHODS

Bacterial and phage strains. *E. coli* C600 *thr leu supE*, from our collection, served as the wild-type strain in this work. *E. coli* K-12 159 *uvrA*, used in the UV irradiation experiments, was also from our collection. Strain K2801 *grpE280* was kindly provided by Hisao Uchida. Strain NK6024 *pheA*::Tn*10* Hfr Hayes, used in cotransduction experiments with the nearby *grpE* locus, was provided by Nancy Kleckner. Strain MF687, carrying the *mutD5* allele isolated by Degnen and Cox (5), was used in phage mutagenesis experiments.

Phage λ *c*I857 was from our collection. Transducing phages λ *c*I857 *grpE*$^+$ and λ *c*I857 *grpE*$^+$ Δ7 (20) were a gift of Hisao Uchida. An *E. coli* DNA library, prepared by partial digestion with *Sau*3A restriction nuclease, in λ vector L47.1 (16) was obtained from Sam Cartinhour. Phage P1L4, used in the transduction studies, was obtained from Lucien Caro.

Media. L broth contained 10 g of tryptone, 5 g of yeast extract, and 5 g of NaCl per liter. The pH was adjusted to 7.4 by the addition of NaOH. T broth was L broth without yeast extract. T agar and L agar plates were prepared from the corresponding broths with the addition of 10 g of agar (Difco Laboratories) per liter. The composition of high-sulfur M9 medium for labeling with [^{35}S]methionine has been described previously (11).

P1 transduction experiments. Phage P1L4 was grown and used in transduction experiments as described by Miller (17).

One- and two-dimensional gel electrophoresis. The procedures used for isoelectric focusing in the first dimension (1.6% [vol/vol] ampholines pH 5 to 7, 0.4% [vol/vol] ampholines pH 3.5 to 10) and sodium dodecyl sulfate (SDS)-polyacrylamide (12.5% [wt/vol]) gel electrophoresis in the second dimension have been described previously (12).

Labeling experiments. The procedure used for labeling UV-irradiated *E. coli* K-12 159 *uvrA* bacteria (45,000 ergs/mm^2 at 254 nm) after infection with various derivative phage strains has been described (11). To preferentially label heat shock proteins, strain C600 *grpE*$^+$ bacteria were grown in M9 high-sulfur medium at 30°C to approximately 3×10^8 cells per ml. A portion (usually 1 ml) was transferred to a 15-ml Corex tube prewarmed at 43°C and labeled with 10 μCi of [^{35}S]methionine (Amersham) between 5 and 15 min after the temperature shift. The bacteria were centrifuged for 1 min in an Eppendorf microfuge, suspended in two-

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dimensional lysis buffer, lysed by three freeze-and-thaw cycles, and stored at −20°C until use.

DNA, RNA, and protein synthesis experiments. Cells were grown at 30°C in high-sulfur M9 medium. Portions (0.5 ml) were distributed into prewarmed (44°C) culture tubes at time zero. Five microliters of label (final concentrations were 4 [29 Ci/mmol], 1.4 [39 Ci/mmol], and 3.3 [1,480 Ci/mmol] μCi of [^{3}H]thymidine [ICN], [^{3}H]uridine [ICN], and [^{35}S]methionine [Amersham], per ml, respectively) were added to each tube at the appropriate times. Following incubation for 2 min, 0.5 ml of 10% cold trichloroacetic acid was added. Samples were chilled on ice prior to further processing. The cells were washed on glass fiber filters (Whatman GF/C) with cold 5% trichloroacetic acid, followed by washes with cold distilled water and then with cold ethanol. The dried filters were counted in a Beckman LS 333 liquid scintillation counter.

RESULTS

Isolation of *grpE* transducing phage. To characterize the *grpE* gene product, we sought to obtain *grpE* point mutations. This was done by growing the λ *c*I857 *grpE*$^+$ Δ7 transducing phage (20) on the *E. coli mutD* strain. The mutagenized phage were plated on a mixture of C600 *grpE*$^+$ and C600 *grpE280* isogenic bacteria (1:5). Small and turbid-looking plaques were tested for growth on both C600 *grpE*$^+$ and C600 *grpE280* bacterial lawns. Phage which grew on C600 *grpE*$^+$ but not on C600 *grpE280* bacteria were presumed to be λ *grpE* mutants. Approximately 20 such phage mutants were isolated, at an estimated frequency of 3×10^{-4}.

Isolation of λ *grpE*$^+$ revertants. Mutant *grpE* phage were plated on C600 *grpE280* bacteria, and revertant plaques, appearing at a frequency of 10^{-5} to 10^{-3}, were purified for one cycle and tested further. The majority of the λ *grpE* mutant phage isolated as described above behaved this way. However, we found that some λ *grpE* phage did not revert (frequency less than 10^{-7}), suggesting that they had large deletions of the *grpE* gene carried on the transducing phage.

***grpE* gene product is a 24,000-M_r protein.** The *grpE* locus was originally identified because a single mutation in it, *grpE280*, blocked phage λ DNA replication at all temperatures (20; our unpublished results). The isolation of a λ *grpE*$^+$ transducing phage and subsequent deletion analysis resulted in the tentative identification of GrpE as a 24,000-M_r, mildly acidic protein (20). We confirmed this conclusion by showing that the presence of this protein in two-dimensional gels correlated with the phenotype of the λ *grpE*$^+$ transducing phage (Fig. 1A, filled arrow). Specifically, the majority of the λ *grpE* derivative phage that we isolated failed to induce or induced substantially less of the 24,000-M_r protein. Infection of UV-irradiated bacteria by λ *grpE40* mutant phage did not induce synthesis of the 24,000-M_r protein (Fig. 1B). The *grpE40* mutation is not suppressible by *supD*, *supE*, or *supF* (17), suggesting that it is not an amber mutation. The production of a fragment suggests that *grpE40* may be a nonsense or frameshift mutation. λ *grpE40R*, a GrpE$^+$ revertant of phage λ *grpE40*, induced the synthesis of normal amounts of the 24,000-M_r protein (Fig. 1C). The ability to induce the synthesis of normal amounts of the 24,000-M_r protein was associated with all GrpE$^+$ revertants of phage λ *grpE*40. All of these results together demonstrated that the *grpE* gene product is the 24,000-M_r acidic protein, as originally suggested by the results of Saito et al. (20).

Isolation of additional λ *grpE*$^+$ transducing phage. An *E. coli* DNA library was prepared by partially digesting *E. coli* DNA with *Sau*3A restriction nuclease and ligating the fragments into the unique *Bam*HI restriction site of the phage λ vector L47.1 (16). By plating this phage library on *grpE280* bacteria, it was found that plaque-formers arose at a frequency of 10^{-4} to 10^{-3}, which is approximately 10,000-fold higher than the expected frequency of occurrence of π mutations in the phage replication gene *P* (20; our unpublished results). We verified that these phages were indeed λ *grpE*$^+$ transducing phage by demonstrating that (i) they induced synthesis of the 24,000-M_r polypeptide after infection of UV-irradiated hosts (data not shown) and (ii) after lysogenization of *grpE280* bacteria, they simultaneously conferred the GrpE$^+$ phenotype for both heteroimmune phage growth and bacterial colony formation at 43°C (data not shown).

GrpE protein is *E. coli* B25.3 heat shock protein. Neidhardt and his colleagues have identified over 17 *E. coli* proteins as belonging to the heat shock family of proteins (19). A prominent member of this group is protein B25.3. Because the size and position in two-dimensional gels of the B25.3 protein were very close to those of the GrpE protein and the DnaK and DnaJ proteins of *E. coli*, which are also necessary for λ DNA replication, have been shown to be heat shock proteins (2, 12), we investigated the possible identity of the GrpE and B25.3 proteins. Fig. 2A shows the two-dimensional electrophoresis pattern of heat-shocked *E. coli* cells. The filled arrow points to the position of the B25.3 protein. Extracts of UV-irradiated bacteria infected with phage λ *grpE*$^+$ Δ7 (Fig. 2B) and extracts of heat-shocked *E. coli* (Fig. 2A) containing approximately equal amounts of radioactivity in the GrpE and B25.3 protein, respectively, were mixed, and their proteins were separated by two-dimensional gel electrophoresis; the GrpE and B25.3 protein spots coincided (Fig. 2C).

The identity of the two proteins was verified in the following way. The ^{35}S-labeled GrpE and B25.3 protein bands were excised from SDS-polyacrylamide gels and partially digested with *Staphylococcus aureus* V8 protease

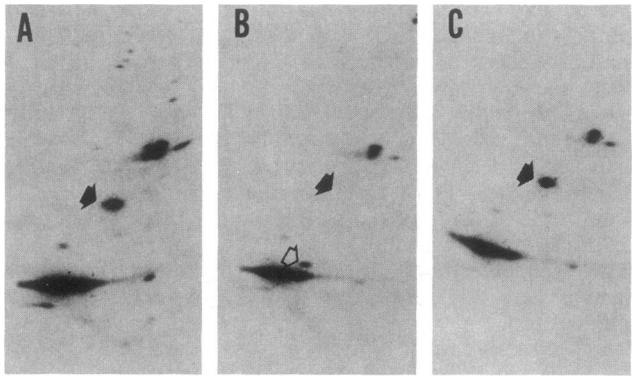

FIG. 1. Two-dimensional gel electrophoresis of UV-irradiated strain 159 *uvrA* bacteria infected with λ *grpE*. (A) λ *grpE*$^+$ Δ7; (B) λ *grpE40*; (C) λ *grpE40R* (revertant). Proteins were labeled with [^{35}S]methionine (30 μCi/ml) for 15 min at 37°C. Only the region of each gel ranging from pH 4.5 to 5.5 (horizontal axis) is shown. Filled arrows indicate the position of the wild-type 24,000-M_r polypeptide. The open arrow indicates the position of a new spot whose synthesis was induced by λ *grpE40* mutant phage (see text for details). In all instances the acidic side of the gel is on the right and the basic side is on the left.

by the method of Cleveland et al. (3). The partial digestion patterns of the GrpE (induced by the λ *grpE*$^+$ transducing phage) and B25.3 (induced after heat shock) proteins were identical (data not shown). We conclude that the GrpE protein is identical to the B25.3 heat shock protein of *E. coli*.

***grpE* gene product required for *E. coli* growth at high temperature.** The *grpE280* bacterial mutant was isolated as a temperature-resistant survivor at 42°C (20). However, upon testing, we found that this strain, K2801 *grpE280*, was unable to form colonies at temperatures above 43.5°C. To prove that the temperature-sensitive phenotype is due to the *grpE280* mutation and not to another mutation caused by the original mutagenesis procedure, we carried out the P1 transduction experiments shown in Table 1. We took advantage of the existence of a transposon Tn*10* insertion in the *pheA* gene to select for transfer of the *grpE*$^+$ and *grpE280* markers between strains. Previously, Saito et al. (20) had shown that the P1 cotransduction frequency between *pheA* and *grpE* is 61%. We confirmed this result by showing that the cotransduction frequency of the Tet^r and GrpE$^+$ markers was 58 to 70% (Table 1). The Tet^r transductants of the crosses shown in Table 1 were further analyzed for the ability to support phage λ growth and the inability to form colonies at 43.5°C. In no case (0 of 200) were we able to separate these two phenotypes. We conclude that the *grpE280* mutation is responsible for the block to both phage growth at all temperatures and bacterial colony formation at high temperature. A possibility that cannot be excluded by these data is that another mutation, tightly linked to *grpE280*, is responsible for the inability to form colonies at high temperature. This possibility was examined through an analysis of Ts$^+$ bacterial revertants. Bacteria carrying the *grpE280* mutation reverted to Ts$^+$ at a frequency of 10^{-7} to 10^{-5} at 43.5°C and at a frequency of 10^{-8} to 10^{-7} at 44.5°C. A substantial fraction (5 of 17) of the Ts$^+$ revertants at 44.5°C simultaneously recovered the ability to propagate phage λ. This result suggests that the *grpE280* mutation is indeed responsible for both phenotypes.

TABLE 1. P1 transduction studies with the *grpE280* mutation[a]

Donor	Recipient	Selected marker	Unselected markers[b]	No.
NK6024 *pheA*::Tn*10*	K2801 *grpE280*	Tet^r	λ$^-$ Ts$^-$	30
			λ$^+$ Ts$^+$	70
			λ$^-$ Ts$^+$	0
			λ$^+$ Ts$^-$	0
K2801 *grpE280* *pheA*::Tn*10*	C600	Tet^r	λ$^-$ Ts$^-$	58
			λ$^+$ Ts$^+$	32
			λ$^-$ Ts$^+$	0
			λ$^+$ Ts$^-$	0

[a] Tetracycline-resistant transductants were selected by incubation for 36 h at 37°C on L agar plates supplemented with 20 μg of tetracycline per ml and 5×10^{-3} M sodium citrate (to prevent P1 reinfection). Inability to propagate phage was tested either by streaking the colonies against phage λ *c*I857 or by spot tests. In all cases it was verified that the λ *c*I857 *grpE*$^+$ transducing phage grew well. The inability to form colonies at high temperature (Ts$^-$) was tested by streaking individual colonies on L agar plates containing tetracycline and incubating at 44°C for 36 h.

[b] λ$^-$, Block to phage λ infection; λ$^+$, susceptibility to phage λ infection.

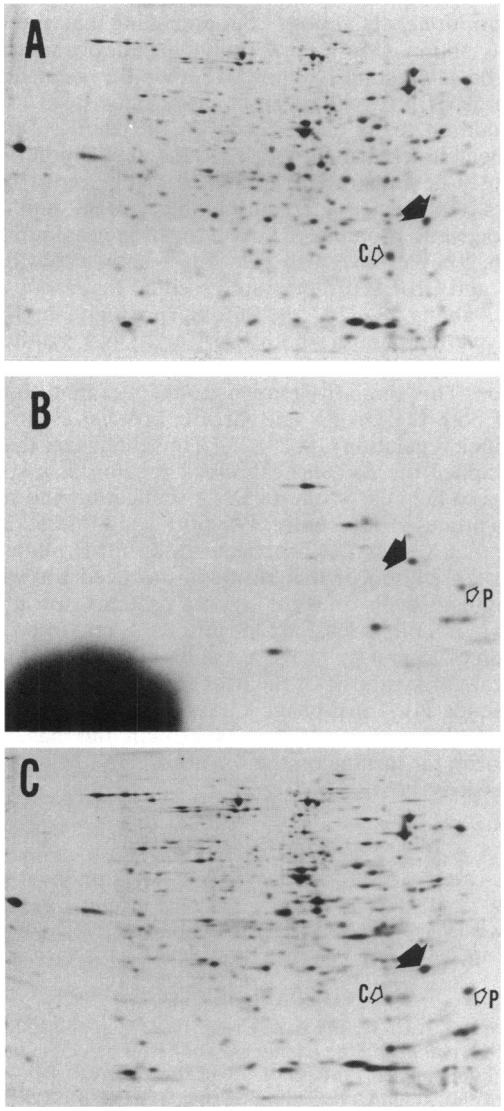

FIG. 2. Two-dimensional gel electrophoresis of heat-shocked C600 *grpE*$^+$ bacteria. (A) Uninfected culture labeled between 5 and 15 min after a shift to 43°C; (B) UV-irradiated cells infected with λ *grpE*$^+$ Δ7, as described in the legend to Fig. 1; (C) coelectrophoresis of a mixture of the extracts shown in panels A and B. Arrows indicate the positions of the GrpE and B25.3 proteins. C, Host-coded protein; P, phage-coded protein.

***grpE280* interferes with both DNA and RNA synthesis.** The rates of synthesis for DNA, RNA, and protein were determined in C600 *grpE*$^+$ and C600 *grpE280* isogenic bacteria at both the permissive (30°C) and nonpermissive (44°C) temperatures. At 44°C the rate of synthesis for DNA and RNA in the *grpE280* mutant declined substantially over a period of 90 min, whereas that of protein did not (Fig. 3). At 30°C, the permissive temperature, the rates of DNA, RNA, and protein synthesis were virtually identical in *grp* and *grpE280* bacteria (data not shown). We conclude that the GrpE protein directly or indirectly affects both DNA and RNA synthesis in *E. coli*, at least at high temperature. Similar observations have been made for mutations in both the *dnaK* (14, 21) and *dnaJ* (27) genes.

The evidence that the *E. coli* heat shock protein B25.3 is identical to the GrpE protein can be summarized as follows. (i) λ *grpE*$^+$ transducing phages, selected as plaque-formers on *grpE280* bacteria, always induced the synthesis of a 24,000-M_r protein; (ii) most of the λ *grpE* mutant phage, isolated as unable to propagate on *grpE280* bacteria, did not induce synthesis of the 24,000-M_r protein; (iii) bona fide B25.3 protein, synthesized by heat-shocked *E. coli* cells, and

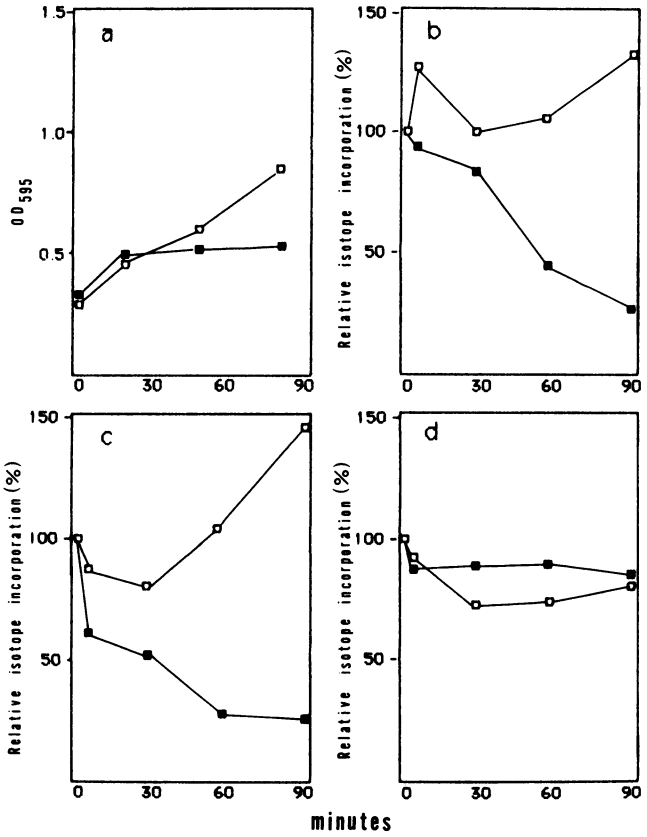

FIG. 3. Rates of DNA, RNA, and protein synthesis in C600 *grpE280*. (a) Rate of cell growth after a shift in temperature from 30 to 44°C at time zero (optical density [OD] at 595 nm); (b) rate of [^{3}H]thymidine incorporation; (c) rate of [^{3}H]uridine incorporation; (d) rate of [^{35}S]methionine incorporation. Percent incorporation is determined relative to incorporation at time zero, designated as 100%. The values at time zero were 2,341 and 4,960 cpm for [^{3}H]thymidine, 65,907 and 76,047 cpm for [^{3}H]uridine, and 321,204 and 297,733 cpm for [^{35}S]methionine for C600 *grpE*$^+$ (□) and C600 *grpE280* (■), respectively.

the GrpE protein induced by λ *grpE*$^+$ transducing phage comigrated in two-dimensional acrylamide gels; and (iv) the GrpE and B25.3 proteins exhibited identical patterns of partial proteolytic products.

DISCUSSION

The *dnaK*, *dnaJ*, and *grpE* genes exhibit a number of similarities. (i) All three were originally discovered because mutations in them interfered with phage λ replication at all temperatures (8, 21, 23). (ii) All three gene products interact with the λ *P* gene replication protein inasmuch as phage mutations mapping in the *P* gene (8, 10, 21, 23) can bypass the bacterial mutation blocks. (iii) All three were shown to code for proteins belonging to the heat shock group of proteins whose expression requires the *htpR* (σ^{32}) gene product (13, 19): *dnaK* codes for a 70,000-M_r protein called B66.0 (12, 19), *dnaJ* codes for a 37,000-M_r heat shock protein called H26.5 (2, 19), and *grpE* codes for a 24,000-M_r protein called B25.3 (19, 20; this work). (iv) All three gene products are essential for *E. coli* growth, at least at high temperature (8, 22, 23; this work). (v) Mutations in all three directly or indirectly interfere with the overall rates of synthesis of both bacterial DNA and RNA at the nonpermissive temperature (14, 21, 22, 27; this work).

Both the DnaK (30) and DnaJ (32) proteins have been purified to homogeneity. The DnaK protein has been shown to exist mostly as a monomer in solution and to possess both nonspecific 5′-nucleotidyl phosphatase (B. Bochner, personal communication; our unpublished data) and autophosphorylating activities (30, 31). In addition, it has been shown to be 48% identical at the amino acid sequence level with the *Drosophila melanogaster* hsp70 (1). The purified DnaJ protein has been shown to exist mostly as a dimer in solution and to bind to both single- and double-stranded DNA without obvious specificity (32). Both proteins have been shown to be absolutely essential for λ DNA replication at all temperatures in an in vitro system (15, 30–32). The level of action of the DnaK and DnaJ proteins in λ DNA replication has been shown to be at a step following the need for the λ O and P replication proteins, the bacterial DnaB protein, and "transcriptional activation," but preceding that of the DnaG primase action (M. Zylicz, unpublished observation). It would be interesting to know whether the need for GrpE protein in λ DNA replication is at the same level.

In addition to its dependence on the DnaK, DnaJ, and GrpE heat shock proteins for its DNA replication, we have previously demonstrated through the isolation of bacterial mutants that phage λ requires the GroES and GroEL morphogenetic proteins of *E. coli* for proper assembly of its head (6, 9). We have also previously shown that both the GroES and GroEL proteins are essential for *E. coli* colony-forming ability at 42°C (9, 26). Surprisingly, at the nonpermissive temperature both RNA and DNA syntheses are affected (26), a result reminiscent of the effect of the *grpE280* mutation. This similarity extends to the fact that the expression of both the GroES and GroEL proteins is also under heat shock regulation (18, 24, 25). Thus it appears that phage λ has tapped the *E. coli* heat shock regulon as a source of proteins to help carry out its DNA replication and morphogenesis processes. Recently, Waghorne and Fuerst (28) have shown that λ does not propagate in an *htpR* mutant host. One interpretation of this result is the need for wild-type intracellular levels of heat shock proteins for proper λ growth in *E. coli*, at least at high temperatures. Interestingly, infection of *E. coli* by phage λ results in a transient increase in the rate of synthesis of the heat shock proteins (reviewed in reference 24). Thus, phage λ has not only adapted some of the heat shock proteins for its growth but has found a mechanism for turning on their synthesis following infection of a sensitive host.

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