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# Interaction of the Heat Shock Protein GroEL of *Escherichia coli* with Single-Stranded DNA-Binding Protein: Suppression of *ssb-113* by *groEL46*

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**Previous studies from our laboratory have shown that an allele of the heat shock protein GroEL (*groEL411*) is able to specifically suppress some of the physiological defects of the single-stranded DNA-binding protein mutation *ssb-1*. A search for additional alleles of the *groE* genes which may act as suppressors for *ssb* mutations has led to the identification of *groEL46* as a specific suppressor of *ssb-113*. It has very little or no effect on *ssb-1* or *ssb-3*. All of the physiological defects of *ssb-113*, including temperature-sensitive growth, temperature-sensitive DNA synthesis, sensitivity to UV irradiation, methyl methanesulfonate, and bleomycin, and reduced recombinational capacity, are restored to wild-type levels. The *ssb-113* allele, however, is unable to restore sensitivity of *groEL46* cells to phage λ. The mechanism of suppression of *ssb-113* by *groEL46* appears to differ from that of *ssb-1* by *groEL411*. The data suggest that GroEL may interact with single-stranded DNA-binding protein in more than one domain.**

The single-stranded DNA-binding protein (SSB) is an essential component in key metabolic reactions of DNA metabolism in bacteria. It plays several important roles in DNA replication, as well as in DNA repair and recombination. The specific roles of SSB in these processes have been discussed in detail in several recent reviews (24, 37, 46, 52). Mutations in the *ssb* gene have proved to be excellent as a means of probing the various functional domains of the SSB protein and have suggested that the defect in SSB-113 lies in an inability to interact properly with other enzymes, while that of SSB-1 lies in an inability to form tetramers (11, 52, 72) or to properly bind DNA in the tetrameric form (7). The use of extragenic suppressors is a useful experimental approach to probe and identify in vivo protein-protein interactions (27). A defect in one protein due to mutation may be suppressed by a compensatory mutation that alters the structure of a protein with which it must interact. The result is a functional double mutant. By using such an approach, we have previously identified an unsuspected interaction between SSB and the heat shock (HSP) or stress protein GroEL (54, 60). A mutation (*groEL411*) was able to phenotypically suppress the temperature-sensitive defect of SSB-1 in DNA replication and to partially restore the defect in DNA repair.

Originally, *groEL* was identified by the inability of phage λ or T4 to grow on strains carrying mutations in this gene (19, 73). Subsequently, it was shown that GroEL is involved in λ phage morphogenesis by providing a scaffold for the assembly of phage heads (31). GroEL was later identified as 1 of 20 different proteins now known to be induced by heat shock or other cellular stresses (19, 20, 57). Indeed, at 30°C, GroEL and GroES account for nearly 1% of the total protein, and after temperature shift to 46°C, this percentage increases to 10 to 12% (25, 29). The GroE proteins are among the major HSPs of *Escherichia coli* (25).

In an effort to continue the investigation of the interaction of SSB and the GroE HSPs and to examine the properties of the *groEL411* allele by itself, we attempted to move the *groEL411* mutation into other genetic backgrounds by phage P1 transduction, by using closely linked tetracycline or kanamycin Tn*10* transposon markers (12, 63) or the ampicillin resistance gene (*ampCp1*) which lies near the *groE* locus (3). However, no transductants could be obtained, indicating that the *groEL411* mutation is lethal in other backgrounds. Consequently, we sought to find other *groE* alleles which could suppress *ssb* mutations. We obtained nine different strains carrying a *groEL* or a *groES* mutation and constructed double mutants with one of these *groE* alleles and either *ssb-1*, *ssb-113*, or *ssb-3*. In the present study, we show that the *groEL46* allele can completely suppress all physiological defects of the *ssb-113* mutation but has no effect on the other *ssb* alleles tested. The *ssb-113* mutation, however, cannot reciprocally suppress the $\lambda^r$ phenotype of *groEL46*. These results strengthen the evidence for a protein-protein interaction between SSB and GroEL proteins. That the defect of SSB-113 is so markedly different from that of SSB-1 suggests that suppression by GroEL46 involves a mechanism different from that of SSB-1 suppression by GroEL411.

## MATERIALS AND METHODS

**Bacterial and phage strains.** The bacterial strains used in this work are listed in Table 1. The *groE* mutations examined included *groES7*, *groES24*, *groES30*, *groES97*, *groEL35*, *groEL46*, *groEL59*, *groEL100*, and *groEL140*. Phage P1 *vir* was obtained from J. Zengel, University of Rochester, and λ phage was obtained from H. Echols, University of California, Berkeley.

**Strain constructions.** The mutant *groE* strains were obtained from the laboratories of G. Walker and C. Georgopoulos (12, 15). All *groE* mutant alleles were transduced by phage P1 *vir* into an *E. coli* W3110 background by the method of Miller (56) by using the linked *purA*::Tn*10* marker. Tetracycline-resistant transductants were selected and subsequently tested for temperature sensitivity and

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TABLE 1. Bacterial strains used

| Strain | Relevant genotype or phenotype | Source or reference |
|---|---|---|
| AB1157 | (F⁻) *thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 mgl-51* λ⁻ *rac hisG4 rfbD1 rpsL31 kdgK51 mtl-1 xyl-5 argE3 thi-1* | B. Bachmann |
| W3110 | (F⁻) IN(*rrnD-rrnE*)*1* λ⁻ | B. Bachmann |
| CG714 | W3110 *galE relA groEL140* | 15 |
| CS196 | AB1157 *lon Δlac-169, sulΔ*::*lacZ* [λ p1(209)] *ssb-3 zjc-1*::Tn*10tet* | I. Tessman |
| RM244 | *proA23 lac-28 tsx-81 trp-30 his-51 rpsL173 ampCp1* Str$^r$ | B. Bachmann |
| RM508 | MG1655 *malF3180*::Tn*10kan* | 63 |
| RM593 | W3110 *purA*::Tn*10tet groEL46* | This study |
| RM598 | W3110 *purA*::Tn*10tet* | This study |
| RM599 | W3110 *purA*::Tn*10tet groEL46 ssb-113* | This study |
| RM626 | W3110 *ssb-3* | This study |
| RM630 | W3110 *ssb-1* | This study |
| RM631 | W3110 *ssb-113* | This study |
| RM644 | W3110 *purA*::Tn*10tet groEL46 ssb-3* | This study |
| RM645 | W3110 *purA*::Tn*10tet groEL46 ssb-1* | This study |
| RM646 | W3110 *purA*::Tn*10tet ssb-113* | This study |
| RM647 | W3110 *malB*::Tn*10kan groEL46 ssb-113* | This study |

resistance to bacteriophage λ. Cotransduction frequencies averaged 50%. When necessary, strains were cured of the transposon by the method of Bochner et al. (5). The presence of the *purA*::Tn*10* transposon had no effect on the behavior of the *ssb* or *groE* alleles.

**Media.** LB broth, LB agar plates, LB medium lacking NaCl (LBNS), and nutrient agar plates were prepared as described previously (56). Because salt can physiologically suppress some *ssb* mutations (52, 64), it was necessary to use LBNS in most assays. Both MacConkey-lactose agar (Difco) and 1% peptone (BBL) with the additions of 0.05 mg of 2,3,5-triphenyl-2H-tetrazolium chloride per ml and 1% lactose (TTC plates) were used to determine recombination frequencies of *lac* mutants. Minimal M9 salts medium with the additions of 50 μg of thymine per ml and 10 μg of thiamine per ml was used in some experiments as indicated.

**Chemicals and antibiotics.** Methyl methanesulfonate (MMS) was purchased from Eastman-Kodak. Bleomycin sulfate, tetrazolium, and antibiotics were obtained from Sigma Chemical Co. The antibiotics were used at the following final concentrations: tetracycline, 20 μg/ml; kanamycin sulfate, 40 μg/ml; streptomycin, 65 μg/ml; and ampicillin, 10 μg/ml. New England Nuclear (DuPont) supplied the [$^3$H]thymidine.

**Efficiency of plating (EOP) at 43°C.** Cells were grown in LBNS to an $A_{595}$ of 0.5 at 32°C, diluted in M9 salts, and plated on LBNS agar in triplicate at 32 and 43°C. The EOP was calculated from the number of CFU at 43°C divided by that at 32°C.

**UV sensitivity.** Qualitative assays for UV sensitivity were performed as follows. Toothpick streaks of colonies to be tested were made across agar plates. One half of the plate was covered with a piece of black paper as a shield. The plate was then irradiated with a germicidal lamp at 20 J/m$^2$, incubated overnight in the dark, and examined for growth on the irradiated side of the plate the following day. For quantitation of UV sensitivity, cells were grown in liquid culture to an $A_{595}$ of 0.5 at 32°C. After collection by centrifugation, they were resuspended in 0.5 volume of M9 salts. A 3- to 5-ml sample was removed and placed in a sterile glass 150-mm petri dish. Cells were irradiated at a rate of 1 J/m$^2$/s. At various intervals, duplicate or triplicate aliquots were removed under a dim yellow light, diluted in M9 salts, plated, and incubated in the dark at 32°C. On the following day, the number of survivors was determined and compared with that of untreated controls. Each experiment was performed at least three times.

**Cell survival after treatment with DNA-damaging agents.** Cells were grown in liquid culture as described above for UV irradiation. Samples of 3 to 5 ml were removed and subjected to one of two treatments as described below. At various intervals, duplicate or triplicate aliquots were removed, quickly diluted in M9 salts, plated, and incubated at 32°C to determine survivors. MMS and bleomycin were used at final concentrations of 38 mM and 10 μg/ml, respectively, according to Hagensee et al. (26).

**Recombination frequencies.** Strain RM244 served as the donor for P1 *vir* transduction of either *lac29* (*lac* mutant) or Str$^r$ markers. Postinfection, the recipient strains were plated on either MacConkey agar or TTC plates and on LB plates containing streptomycin and were incubated overnight at 32°C.

Recombination frequencies were determined from the number of Str$^r$ CFUs or *lac* mutant CFUs divided by the total number of recipient cells on LB plates. At least three independent experiments were performed for each marker.

**DNA synthesis.** Cells were grown in 20 ml of LBNS in a 250-ml flask at 32°C to an $A_{595}$ of 0.20 to 0.25. Beginning at 30 min before temperature shift (−30), triplicate 0.2-ml aliquots were removed, placed into test tubes (10 by 75 mm), and pulse-labeled for 5 min at 32°C with [$^3$H]thymidine at a concentration of 1 μCi/ml. At time zero, 5 ml of cells was removed from the flask by using a prewarmed pipette and transferred to a 125-ml flask in the 44°C water bath. (The water bath was maintained at 44°C to ensure that the temperature within the assay tubes was at least 43°C.) The culture was permitted to equilibrate for 1 min at the higher temperature, and then 0.2-ml aliquots were transferred to test tubes and pulse-labeled for DNA synthesis as described above. At the higher temperature, pulse-labeling was carried out for 3 min (21). Synthesis was stopped with 0.5 ml of cold 10% trichloroacetic acid on ice. The acid-precipitable DNA was collected on glass fiber filters and washed, and the radioactivity was determined by scintillation counting.

**Sensitivity to phage λ.** A fast, qualitative test for phage λ sensitivity was carried out by a streak assay. A sample of 20 μl of a high-titer λ phage stock (~$10^{11}$ PFU/ml) was streaked down the center of a petri dish containing nutrient agar.

TABLE 2. Specificity of suppression of the temperature sensitivity of the *ssb-113* mutation by *groEL46*

| Strain genotype | EOP[a] at 43°C |
|---|---|
| Wild type | 1.01 |
| *groEL46* | 0.68 |
| *ssb-113* | 0.0000097 |
| *ssb-113 groEL46* | 1.05 |
| *ssb-1* | 0.0038 |
| *ssb-1 groEL46* | 0.0027 |
| *ssb-3* | 0.0015 |
| *ssb-3 groEL46* | 0.00018 |

[a] EOP is the number of CFU 43°C divided by the number of CFU at 30°C.

Strains to be tested were streaked from left to right across the plate through the λ phage and then incubated overnight. Strains resistant to the phage showed good growth across the entire plate, while those sensitive to the phage showed reduced or no growth to the right of the λ streak. To confirm phage sensitivity and for quantitative assays, the titer of the λ phage was determined by the method of Maniatis et al. (47). A 0.1-ml aliquot of diluted phage stock was added to 0.1 ml of indicator cells grown overnight on LB agar plus 0.2% maltose and incubated at 37°C. After 15 min, 2.5 ml of top agar was added and the mixture was poured onto LB plates. The plates were examined for plaques after overnight incubation at 37°C.

## RESULTS

**Construction of *ssb groE* double mutants and identification of a suppressor of *ssb-113*.** We constructed 27 potential suppressor strains by independently moving nine different *groE* mutations by phage P1 transduction into strains carrying either *ssb-1*, *ssb-113*, or *ssb-3* alleles. All of the *ssb* mutants are temperature sensitive for growth and sensitive to UV. The *purA*::Tn*10* tetracycline resistance marker, which is ~50% cotransducible with *groE*, was used for selection of this marker. Some of the *groE* mutants are temperature sensitive, but all are resistant to phage λ. Thus, the presence of a *groE* allele was determined qualitatively by a λ streak assay and then verified by testing for the inability to form λ plaques in top agar. All 27 strains were subsequently tested for their ability to grow on LBNS plates at restrictive temperatures (43°C) and for resistance to UV irradiation by using the qualitative UV screening assay. One of these strains (RM599, carrying the *groEL46* allele) showed phenotypic suppression of the *ssb-113* mutation. The presence of both *ssb-113* and *groEL46* alleles in this strain was confirmed by independently transducing out each allele from RM599. By using a Tn*10* Kan$^r$ marker located at *malB* (63) which cotransduces ~8% with *ssb*, *ssb-113* was transduced into W3110, selecting for Kan$^r$. Of the transductants screened for UV sensitivity, 7.9% were UV sensitive and all of these were temperature sensitive. Similarly, P1 transduction, by using the linked *purA*::Tn*10* Tet$^r$ marker and testing for λ$^r$, indicated that ~50% of the Tet$^r$ transductants were λ$^r$.

***groEL46* supresses the temperature sensitivity of *ssb-113* and is allele specific.** The suppression of temperature sensitivity of *ssb-113* by *groEL46* is shown in Table 2. At 43°C, the EOP of *ssb-113* cells is $<10^{-5}$, but *groEL46* restores this to wild-type levels. The effect is allele specific, since neither the *ssb-1* nor the *ssb-3* strains show increased survival at 43°C in the presence of *groEL46*. In fact, the *groEL46* mutation reduces the EOP slightly in a W3110 background on LBNS plates (Table 2), and the double mutants, *ssb-1 groEL46* and *ssb-3 groEL46*, also have slightly lowered EOPs than the corresponding strain carrying the *ssb* allele only.

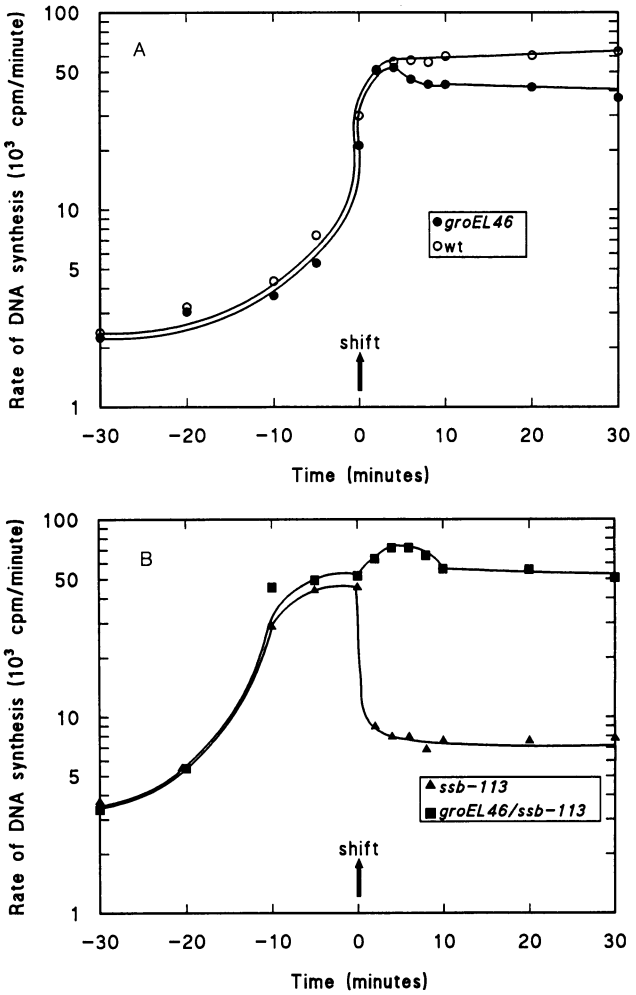


FIG. 1. Rate of DNA synthesis after temperature shift in various strains. Cells were grown to an $A_{595}$ of 0.25 at 32°C. At the times indicated, 0.2-ml aliquots were pulse-labeled with 1 μCi of [$^3$H]thymidine per ml for 5 min (32°C) or 3 min (44°C). The temperature shift occurred at time zero (arrow). (A) ○, RM598 (wild type); ●, RM593 (*groEL46*). (B) ▲, RM646 (*ssb-113*); ■, RM599 (*ssb-113 groEL46*).

**Suppression of temperature-sensitive DNA synthesis of *ssb-113* by *groEL46*.** The *ssb-113* mutation renders the cell defective in DNA synthesis at elevated temperatures. Upon a shift to restrictive temperatures, DNA synthesis drops by an order of magnitude within a minute. We have examined the effects of *groEL46* on DNA synthesis, as shown in Fig. 1. Upon a temperature shift to 44°C, wild-type cells showed a leveling off in DNA synthesis but continued their elevated rate of synthesis of DNA. The *groEL46* strain showed a slight decrease in DNA synthesis, compared with that of controls, but a continued high level of [$^3$H]thymidine incorporation (Fig. 1A). The *ssb-113* strain, however, showed an

immediate, precipitous drop in DNA synthesis after transfer (Fig. 1B), which is consistent with previous studies (53). The *groEL46 ssb-113* double mutant showed an immediate, slight rise in DNA synthesis upon temperature shift, and then continued synthesis at control levels followed. The initial increase in DNA synthesis was reproducibly seen and may correspond to a heat-shock-induced increase in GroEL46 protein. Although the experiment shown in Fig. 1 indicates a slight difference in the initial rate of incorporation between wild-type and *ssb-113* cells, this difference is not significant since some variation was observed from experiment to experiment. However, the important observation, i.e., the pattern of incorporation after temperature shift, was found in all experiments. It is interesting that the *groEL46* suppression of *ssb-113* DNA synthesis is markedly different from *groEL411* suppression of *ssb-1*. In the latter case, upon temperature shift, the rate of DNA synthesis immediately dropped, as in *ssb-1* cells, but then recovered after 4 to 5 min (54, 60).

**Effect of *groEL46* on DNA repair in *ssb-113* cells.** While the *ssb-113* mutation renders cells defective in DNA synthesis only at elevated temperatures, these cells are defective in DNA repair at all temperatures as evidenced by their UV sensitivity (11, 21, 34, 42, 52, 55, 70). We have, therefore, examined the influence of *groEL46* on *ssb-113* UV sensitivity at nonrestrictive temperatures. As shown in Fig. 2A, the *groEL46* suppressor completely restored *ssb-113* cells to wild-type levels of UV resistance. Again, as with temperature sensitivity, this effect is allele specific, since *groEL46* had only a small effect on the UV sensitivity of *ssb-1* cells (Fig. 2B) and absolutely no effect on *ssb-3* cells (Fig. 2C). It is also interesting to note that the *groEL46* suppression of *ssb-113* UV sensitivity is completely effective, whereas suppression of UV sensitivity of *ssb-1* by *groEL411* is only partially effective (53a, 54).

Two other DNA-damaging agents were also examined. The *ssb-113* cells are sensitive to both MMS and bleomycin, although to a much lesser degree than to UV irradiation (Fig. 3 and 4). These chemical agents induce damage which is repaired by excision mechanisms, resulting in short-patch repair. In *E. coli*, SSB is not known to play a direct role in repair via these pathways. Sensitivity to these agents may result from the accumulation of single-stranded DNA after damage and the inability of mutant SSBs to protect this DNA from nuclease attack as efficiently as wild-type SSB does (52). For MMS, the sensitivity of *groEL46* cells is similar to that of wild-type cells. The *groEL46* suppressor completely abolishes the MMS sensitivity of *ssb-113* cells (Fig. 3). The effects of bleomycin, shown in Fig. 4, are rather interesting. Both *ssb-113* and *groEL46* alleles independently resulted in a limited sensitivity to bleomycin, and the sensitivity curves are roughly parallel. The two alleles together, however, mutually abolished the bleomycin sensitivity of each allele alone.

**Effect of *groEL46* on recombination.** While the most dramatic effects of the *ssb-113* and *ssb-1* mutations are on DNA replication and repair (10, 21, 34, 42, 50, 51, 53, 55, 70), these DNA-binding protein mutations also exhibit a less dramatic but significant decrease in recombination (21, 23). We have examined the recombination frequency of two different genes in the *ssb-113* and suppressor strains, as assayed by phage P1 transduction. In comparison to wild-type cells, the *ssb-113* mutation reduces recombination of $Str^r$ and *lac* mutant recombinants by about an order of magnitude (Table 3). The presence of *groEL46* increases recombination back to wild-type levels. Thus, the *groEL46* suppressor is capable

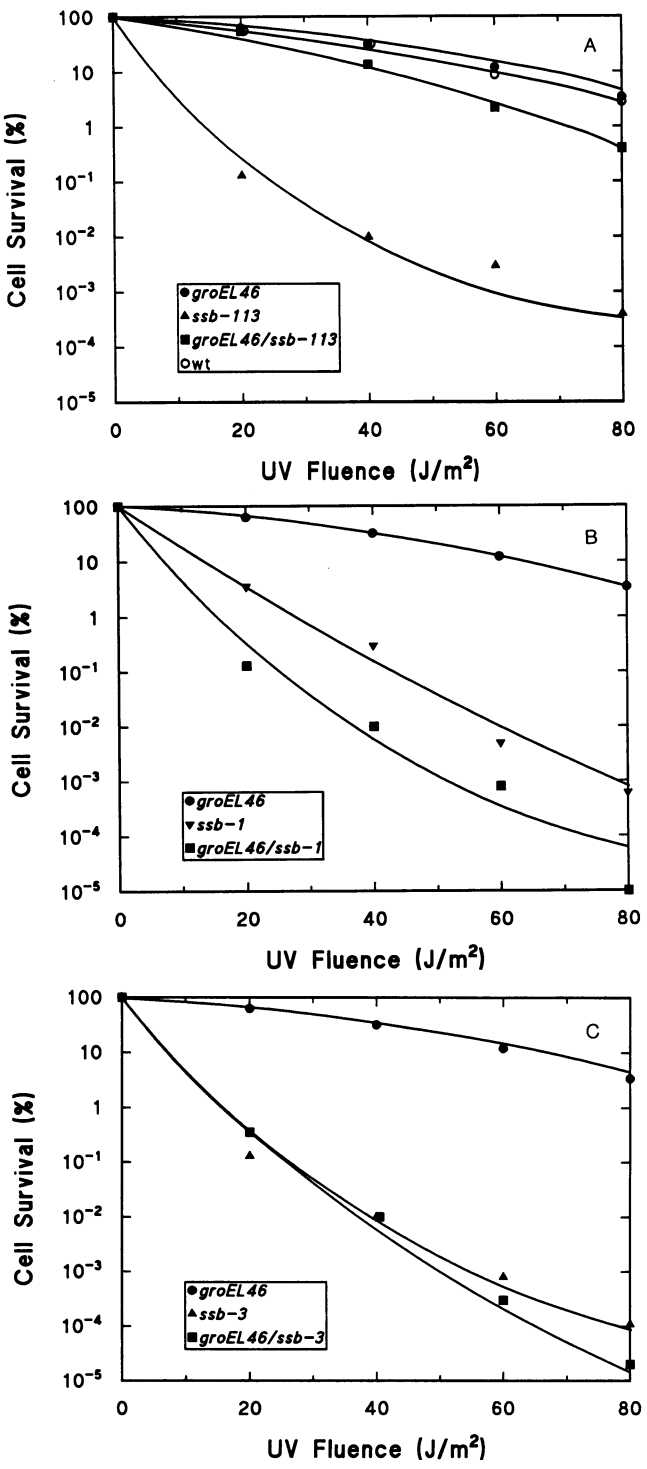


FIG. 2. Cell survival after UV irradiation. Cells were grown and prepared as described in Materials and Methods and irradiated at 1 J/m²/min. Aliquots were removed at various intervals, diluted, and plated to determine survivors. (A) *ssb-113* series. Symbols: ○, RM598 (wild type); ●, RM593 (*groEL46*); ▲, RM631 (*ssb-113*); ■, RM599 (*ssb-113 groEL46*). (B) *ssb-1* series. Symbols: ●, RM593 (*groEL46*); ▼, RM630 (*ssb-1*); ■, RM645 (*ssb-1 groEL46*). (C) *ssb-3* series. Symbols: ●, RM593 (*groEL46*); ▲, RM626 (*ssb-3*); ■, RM644 (*ssb-3 groEL46*).

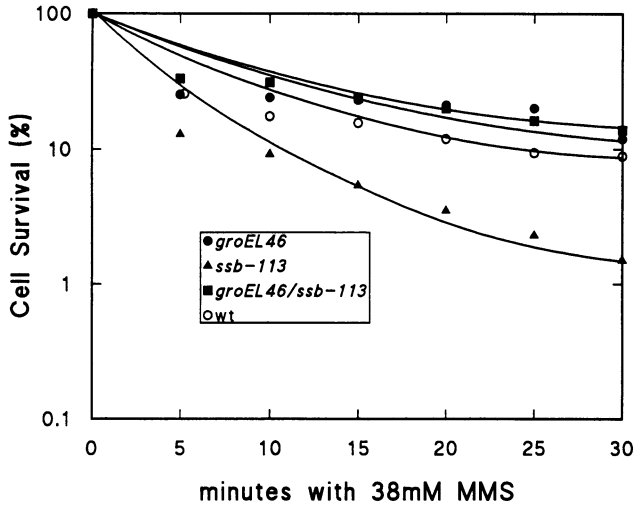


FIG. 3. Sensitivity to MMS. Cells were prepared as described in Materials and Methods and exposed to 38 mM MMS. At various times intervals, aliquots were removed, diluted, and plated to determine survivors. Symbols: ○, RM598 (wild type); ●, RM593 (*groEL46*); ▲, RM631 (*ssb-113*); ■, RM599 (*ssb-113 groEL46*).

of suppressing defects of *ssb-113* in all three of its major functional areas, i.e., replication, repair, and recombination.

***ssb-113* does not suppress the λ growth defect of *groEL46*.** While *groEL46* is able to suppress all of the defects of *ssb-113*, there does not seem to be any reciprocal effect with respect to the ability of *groEL46* to grow λ phage. Although some *groEL* alleles are temperature sensitive, *groEL46* is not (Table 2). However, none of the *groEL* mutations allowed growth of λ phage at any temperature. That no plaques were visible in top agar shows that even the double mutant *ssb-113 groEL46* was unable to support λ growth. Thus, *ssb-113* was unable to correct this defect of *groEL46*.

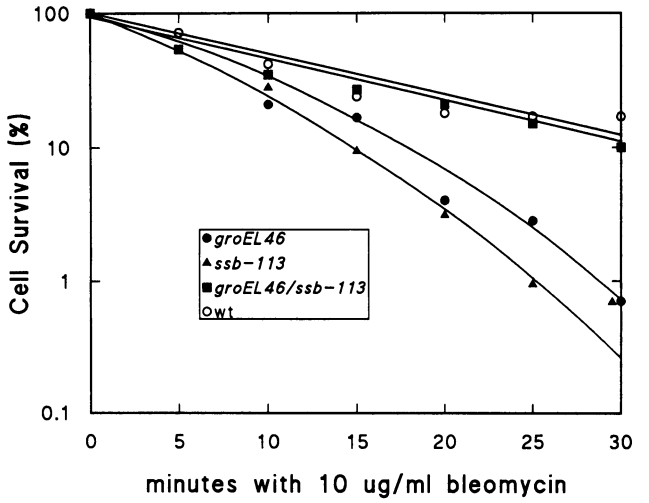


FIG. 4. Sensitivity to bleomycin. Cells were prepared as described in Materials and Methods and exposed to 10 μg of bleomycin per ml. At various times intervals, aliquots were removed, diluted, and plated to determine survivors. Symbols: ○, RM598 (wild type); ●, RM593 (*groEL46*); ▲, RM631 (*ssb-113*); ■, RM599 (*ssb-113 groEL46*).

TABLE 3. Enhancement of recombination[a] in *ssb-113* cells by *groEL46*

| Recipient strain | Genotype | Frequency of recombination | |
|---|---|---|---|
| | | Str[r] recombinants | *lac* mutant recombinants |
| RM598 | *ssb*$^+$ *groEL*$^+$ | $3.3 \times 10^{-7}$ | $4.2 \times 10^{-5}$ |
| RM593 | *ssb*$^+$ *groEL46* | $2.5 \times 10^{-7}$ | $4.6 \times 10^{-5}$ |
| RM631 | *ssb-113 groEL*$^+$ | $0.18 \times 10^{-7}$ | $0.36 \times 10^{-5}$ |
| RM599 | *ssb-113 groEL46* | $4.1 \times 10^{-7}$ | $3.9 \times 10^{-5}$ |

[a] Assayed by phage P1 transduction.

The only observed influence of *ssb-113* on *groEL* was the mutual suppression of bleomycin sensitivity described above (Fig. 4). The possible suppressor effect of *ssb-113* on the *umuCD* mutagenesis defect of *groEL46* has not yet been examined.

## DISCUSSION

**Physiological roles of HSPs.** HSPs or stress proteins have attracted a great deal of interest in recent years, as they have been found in all organisms examined, including thermophiles (43). Some of these HSPs show sequence homology as high as 50% in organisms ranging from bacteria to humans (20, 28, 59, 62, 73). This remarkable conservation indicates that these proteins play major roles in the physiology of all cells. In *E. coli*, there are at least 20 different polypeptides now known to be synthesized after heat shock, and many of them have been characterized (13, 19, 20, 25, 30, 57). Of these, GroEL, belonging to the HSP60 family, is one of the most abundant and interacts with GroES (HSP10) (65). Although GroEL is inducible by elevated temperatures, it is required at all temperatures (16, 74).

Until quite recently, it has been thought that the primary sequence of a polypeptide was necessary and sufficient to determine secondary structure. Folding of the protein into a functional conformation was believed to occur spontaneously until the lowest free-energy state was attained (13). However, it has now been shown that such spontaneous folding is a very slow process for most proteins. Within the cell, proteins called chaperonins or foldases assist in and are essential to the process (13, 20, 30). This, in fact, is likely to be the normal, primary function of HSPs which, upon thermal stress, are induced to higher levels to prevent heat-induced denaturation and/or to help refold proteins. The GroEL HSP has been reported to facilitate folding of citrate synthetase (6), β-lactamase (4, 38), and chloramphenicol acetyltransferase (38) from *E. coli*, and it is involved in its own multimeric assembly (44). Rubisco from the photosynthetic prokaryote *Rhodospirillum rubrum* (22) also interacts with this *E. coli* HSP. GroEL has been shown to renature λ repressor (17) and dihydrofolate reductase (68). Remarkably, the *E. coli* GroEL protein is capable of assisting in the folding of eukaryotic organelle proteins, including rubisco of chloroplasts (18, 28) and rhodanese of mitochondria (39, 48). The GroE proteins are also involved in protein transport mechanisms (1, 2, 4, 36, 40, 58).

**Interaction of SSB and GroEL.** With the present study, we have now identified two *groEL* alleles that demonstrate allele-specific suppression of *ssb* mutations. This raises several interesting questions with regard to the mechanism(s) of suppression and to whether GroEL participates in replication, repair, or recombination. It should be empha-

sized that the data presented here suggest but do not prove a direct interaction between SSB and GroEL. Indeed, there is no evidence that wild-type GroEL is required for proper SSB folding, as indicated by the following observations: (i) overproduction of GroEL or GroES does not restore mutant SSB functions (37a); (ii) wild-type SSB is functional in all five *groEL* mutants tested, although it should be noted that an appropriate *groEL* mutation may not have been found to demonstrate this; (iii) GroEL does not bind to an SSB-affinity column (57a); and (iv) SSB is capable of spontaneously renaturing after heating, forming functional tetramers (51).

**Does GroEL participate in recombination or repair?** There is no evidence for participation of GroEL in recombination. The suppression of recombinational defects of *ssb-113* by *groEL46* described here is the first suggestion of such a role, although, again, it is most likely an indirect effect. Other reports have provided evidence that GroE proteins play an indirect role in SOS repair. Under conditions in which the SOS operon was not inducible, Weigle reactivation of UV-irradiated phages S13 and λ were enhanced by increasing the expression of the *groE* genes (9, 45). Moreover, mutations in either *groEL* or *groES* resulted in a nonmutable phenotype even when the SOS regulon was induced (12). Suppressor studies suggest possible interactions of GroEL or GroES with the *umuDC* gene products which are required for SOS-induced mutagenesis (12). The results reported in the present study indicate that GroEL46 restores the DNA repair defect of SSB-113 to normal, but, again, this is probably an indirect effect.

**Does GroEL participate in DNA replication?** It is now established that three *E. coli* heat shock proteins, DnaJ, DnaK, and GrpE, participate in replication of phage λ, phage P1, and plasmid DNAs (8, 14, 19, 32, 35, 41, 49, 66, 71). However, a direct role for GroEL or GroES in DNA replication has not been observed. Wada and Itikawa (69) found that the *groES131* mutant was temperature sensitive for both replication and transcription. Indirect evidence for the participation of the GroE proteins in replication has been suggested from the observation that overproduction of wild-type GroEL and GroES can suppress certain *dnaA* mutations (15, 33, 67). We have shown that the replication defect of the *ssb-1* mutation can be suppressed by *groEL411* (54, 60) and, in this report, that of the *ssb-113* allele can be suppressed by *groEL46*.

**What is the mechanism of GroEL suppression of mutant SSB proteins?** It is interesting to compare and contrast the effects of the two different *groEL* suppressors on the two *ssb* mutations. The location of the mutations in *groEL* is not yet known and awaits sequence analysis. Thus, there is no current information as to whether the two mutations lie in the same functional domain of the GroEL protein. With the ability of GroEL to interact with so many diverse proteins (as discussed above), it would not be surprising to find more than one functional domain.

Considerably more is known of the defects in the mutant SSB proteins. The *ssb-1* mutation lies at amino acid 55 (His → Tyr) which is in the DNA-binding and subunit interaction domains (52, 72). The SSB-1 protein dissociates into monomers at the restrictive temperature (72) or fails to form functional tetramers (7). We have suggested (52, 54, 60) that the mechanism of action of GroEL411 protein may be one of the following: (i) promotion of tetramer formation by acting as a scaffold for proper folding and alignment; (ii) sequestering of SSB-1 monomers and increasing the local concentration, thereby shifting the equilibrium in favor of tetramers; or (iii) prevention of the unfolding and subsequent dissociation of SSB-1 that occurs at 43°C. Only the replication defect of *ssb-1* is completely suppressed by *groEL411*. In contrast, the *ssb-113* defect is different. The SSB-113 protein forms normal tetramers and binds well to DNA. The mutation lies in the penultimate amino acid (176 Pro → Ser) which is in a region highly conserved among a variety of DNA-binding proteins (61). As proline residues form bends in polypeptide chains, such a change to a serine could have serious consequences for protein folding. It is thought that this domain is involved in protein-protein interactions which are disrupted only at the restrictive temperature for DNA replication but at all temperatures for repair and recombination. Thus, GroEL46 may interact with this C-terminal domain to properly fold and/or refold the SSB-113 protein by virtue of its foldase activity. The *groEL46* allele suppresses all defects of *ssb-113*. The exact mechanism of suppression awaits further experimentation, but the interaction of GroEL with SSB may prove to be an extremely useful one for mechanistic studies of how chaperonins like GroEL and GroES bind to various protein surfaces to facilitate folding or refolding (73). Whether GroEL plays more than just an indirect role in DNA replication is certainly a possibility, but it remains to be demonstrated.

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## REFERENCES

1. **Alder, G. M., B. M. Austen, C. L. Bashford, A. Mehlert, and C. A. Pasternak.** 1990. Heat shock proteins induce pores in membranes. Biosci. Rep. **10:**509–518.
2. **Altman, E., C. A. Kumamoto, and S. D. Emr.** 1991. Heat-shock proteins can substitute for SecB function during protein export in *Escherichia coli*. EMBO J. **10:**239–245.
3. **Bachmann, B.** 1990. Linkage map of *Escherichia coli* K-12, edition 8. Microbiol. Rev. **54:**130–197.
4. **Bochkareva, E. S., N. M. Lissin, and A. S. Girshovich.** 1988. Transient association of newly synthesized unfolded proteins with the heat-shock GroEL protein. Nature (London) **336:**254–257.
5. **Bochner, B. R., H. C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143:**926–933.
6. **Buchner, J., M. Schmidt, M. Fuchs, R. Jaenicke, R. Rudolph, F. Schmid, and T. Kiefhaber.** 1991. GroE facilitates refolding of citrate synthase by suppressing aggregation. Biochemistry **30:**1586–1591.
7. **Bujalowski, W., and T. M. Lohman.** 1991. Monomer-tetramer equilibrium of the *Escherichia coli ssb-1* mutant single strand binding protein. J. Biol. Chem. **266:**1616–1626.
8. **Bukau, B., and G. C. Walker.** 1989. Δ*dnaK52* mutants of *Escherichia coli* have defects in chromosome segregation and plasmid maintenance at normal growth temperatures. J. Bacteriol. **171:**6030–6038.
9. **Calsau, P., and S. Salles.** 1991. Heat-inducible reactivation of UV-damaged bacteriophage λ. Mol. Gen. Genet. **226:**113–119.
10. **Chase, J. W., J. J. L'Italien, J. B. Murphy, E. K. Spicer, and K. R. Williams.** 1984. Characterization of the *Escherichia coli* SSB-113 mutant single-stranded DNA-binding protein. Cloning of the gene, DNA and protein sequence analysis, high pressure liquid chromatography peptide mapping, and DNA-binding studies. J. Biol. Chem. **259:**805–814.
11. **Chase, J. W., and K. R. Williams.** 1986. Single-stranded DNA binding proteins required for DNA replication. Annu. Rev. Biochem. **55:**103–136.

12. **Donnelly, C. E., and G. C. Walker.** 1989. *groE* mutants of *Escherichia coli* are defective in *umuDC*-dependent UV mutagenesis. J. Bacteriol. **171:**6117–6125.
13. **Ellis, R. J., and S. M. van der Vies.** 1991. Molecular chaperones. Annu. Rev. Biochem. **60:**321–347.
14. **Ezaki, B., T. Ogura, H. Mori, H. Niki, and S. Hiraga.** 1989. Involvement of DnaK protein in mini-F plasmid replication: temperature-sensitive *seg* mutations are located in the *dnaK* gene. Mol. Gen. Genet. **281:**183–189.
15. **Fayet, O., J.-M. Louarn, and C. Georgopoulos.** 1986. Suppression of the *Escherichia coli dnaA46* mutation by amplification of the *groES* and *groEL* genes. Mol. Gen. Genet. **202:**435–445.
16. **Fayet, O., T. Ziegelhoffer, and C. Georgopoulos.** 1989. The *groES* and *groEL* heat shock gene products of *Escherichia coli* are essential for bacterial growth at all temperatures. J. Bacteriol. **171:**1379–1385.
17. **Gaitanaris, G. A., A. G. Papavassiliou, P. Rubock, S. J. Silverstein, and M. E. Gottesman.** 1990. Renaturation of denatured λ repressor requires heat shock proteins. Cell **61:**1013–1020.
18. **Gatenby, A. A., and R. J. Ellis.** 1990. Chaperone function: the assembly of ribulose bisphosphate carboxylase oxygenase. Annu. Rev. Cell Biol. **6:**125–149.
19. **Georgopoulos, C., D. Ang, K. Liberek, and M. Zylicz.** 1990. Properties of the *Escherichia coli* heat shock proteins and their role in bacteriophage λ growth, p. 191–221. *In* R. I. Morimoto, A. Tissières, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Georgopoulos, C., D. Ang, A. Maddock, S. Raina, B. Lipinska, and M. Zylicz.** 1990. Heat shock response of *Escherichia coli*, p. 405–419. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington, D.C.
21. **Glassberg, J., R. R. Meyer, and A. Kornberg.** 1979. Mutant single-strand binding protein of *Escherichia coli*: genetic and physiological characterization. J. Bacteriol. **140:**14–19.
22. **Goloubinoff, P. A., A. A. Gatenby, and G. H. Lorimer.** 1989. GroE heat-shock proteins promote assembly of foreign prokaryote ribulose biphosphate carboxylase oligomers in *Escherichia coli*. Nature (London) **337:**44–47.
23. **Golub, E. I., and K. B. Low.** 1983. Indirect stimulation of genetic recombination. Proc. Natl. Acad. Sci. USA **80:**1401–1405.
24. **Greipel, J., C. Urbanke, and G. Maass.** 1989. The single-stranded DNA binding protein of *Escherichia coli*: physiochemical properties and biological functions. *In* W. Saenger and U. Heinemann (ed.), Protein-nucleic acid interactions. CRC Press, Inc., Boca Raton, Fla.
25. **Gross, C. A., D. B. Straus, J. W. Erickson, and T. Yura.** 1990. The function and regulation of heat shock proteins in *Escherichia coli*, p. 167–189. *In* R. I. Morimoto, A. Tissières, and C. Georgopoulos (ed.), Stress proteins in biology and medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
26. **Hagensee, M. E., S. K. Bryan, and R. E. Moses.** 1987. DNA polymerase III requirement for repair of DNA damage caused by methyl methanesulfonate and hydrogen peroxide. J. Bacteriol. **169:**4608–4613.
27. **Hartman, P. E., and J. R. Roth.** 1973. Mechanisms of suppression. Adv. Genet. **17:**1–105.
28. **Hemmingsen, S. M., C. Woolford, S. M. van der Vies, K. Tilly, D. T. Dennis, C. P. Georgopoulos, R. W. Hendrix, and R. J. Ellis.** 1988. Homologous plant and bacterial proteins chaperone oligomeric protein assembly. Nature (London) **333:**330–334.
29. **Herendeen, S. L., R. A. VanBogelen, and F. C. Neidhardt.** 1979. Levels of major proteins of *Escherichia coli* during growth at different temperatures. J. Bacteriol. **139:**185–194.
30. **Hightower, L. E.** 1991. Heat shock, stress proteins, chaperones, and proteotoxicity. Cell **66:**191–197.
31. **Hohn, T., B. Hohn, A. Engel, M. Wurtz, and P. R. Smith.** 1979. Isolation and characterization of the host protein GroE involved in bacteriophage lambda assembly. J. Mol. Biol. **129:**359–373.
32. **Hwang, D. S., E. Crooke, and A. Kornberg.** 1990. Aggregated dnaA protein is dissociated and activated for DNA replication by phospholipase or dnaK protein. J. Biol. Chem. **265:**19244–19248.
33. **Jenkins, A. J., J. B. March, I. R. Oliver, and M. Masters.** 1986. A DNA fragment containing the *groE* genes can suppress mutations in the *Escherichia coli dnaA* gene. Mol. Gen. Genet. **202:**446–454.
34. **Johnson, B. F.** 1984. Two-dimensional electrophorectic analysis of the regulation of SOS proteins in three *ssb* mutants. Arch. Microbiol. **138:**106–112.
35. **Kawasaki, Y., C. Wada, and T. Yura.** 1991. Mini-F plasmid mutant able to replicate in the absence of $\sigma^{32}$: mutations in the *repE* coding region producing hyperactive initiator protein. J. Bacteriol. **173:**1064–1072.
36. **Kusukawa, N., T. Yura, C. Ueguchi, Y. Akiyama, and K. Ito.** 1989. Effects of mutations in heat-shock genes *groES* and *groEL* on protein export in *Escherichia coli*. EMBO J. **8:**3517–3521.
37. **Kowalczykowski, S. C.** 1991. Biochemistry of genetic recombination: energetics and mechanism of DNA strand exchange. Annu. Rev. Biophys. Biophys. Chem. **20:**539–575.
37a. **Laine, P. S., and R. R. Meyer.** Unpublished observations.
38. **Laminet, A. A., T. Ziegelhoffer, C. Georgopoulos, and A. Plückthun.** 1990. The *Escherichia coli* heat shock proteins GroEL and GroES modulate the folding of the β-lactamase precursor. EMBO J. **9:**2315–2319.
39. **Landry, S. J., and L. M. Gierasch.** 1991. The chaperonin GroEL binds a polypeptide in an α-helical conformation. Biochemistry **30:**7359–7362.
40. **Lecker, S., R. Lill, T. Ziegelhoffer, C. Georgopoulos, P. J. Bassford, Jr., C. A. Kumamoto, and W. Wickner.** 1989. Three pure chaperone proteins of *Escherichia coli*—SecB, trigger factor and GroEL—form soluble complexes with precursor proteins *in vitro*. EMBO J. **9:**2703–2709.
41. **Liberek, K., J. Marszalek, D. Ang, C. Georgopoulos, and M. Zylicz.** 1991. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc. Natl. Acad. Sci. USA **88:**2874–2878.
42. **Lieberman, H. B., and E. M. Witkin.** 1983. DNA degradation, UV sensitivity and SOS-mediated mutagenesis in strains of *Escherichia coli* deficient in single-strand DNA binding protein: effects of mutations and treatments that alter levels of exonuclease V or RecA protein. Mol. Gen. Genet. **190:**92–100.
43. **Lindquist, S., and E. A. Craig.** 1988. The heat-shock proteins. Annu. Rev. Genet. **22:**631–677.
44. **Lissin, N. M., S. Venyaminov, S. Yu, and A. S. Girshovich.** 1990. (Mg-ATP)-dependent self-assembly of molecular chaperone GroEL. Nature (London) **348:**339–342.
45. **Liu, S.-K., and I. Tessman.** 1990. *groE* genes affect SOS repair in *Escherichia coli*. J. Bacteriol. **172:**6135–6138.
46. **Lohman, T. M., and W. Bujalowski.** 1989. *Escherichia coli* single strand binding protein: multiple single-stranded DNA binding modes and cooperativities, p. 131–168. *In* A. Revzin (ed.), The biology of nonspecific DNA-protein interactions. CRC Press, Inc., Boca Raton, Fla.
47. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
48. **Mendoza, J. A., E. Rogers, G. H. Lorimer, and P. M. Horowitz.** 1991. Chaperonins facilitate the *in vitro* folding of monomeric mitochondrial rhodanese. J. Biol. Chem. **266:**13044–13049.
49. **Mensa-Wilmot, K., R. Seaby, C. Alfano, M. S. Wold, B. Gomes, and R. McMacken.** 1989. Reconstitution of a nine-protein system that initiates bacteriophage λ DNA replication. J. Biol. Chem. **264:**2853–2861.
50. **Meyer, R. R., J. Glassberg, and A. Kornberg.** 1979. An *Escherichia coli* mutant defective in single-strand binding protein is defective in DNA replication. Proc. Natl. Acad. Sci. USA **76:**1702–1705.
51. **Meyer, R. R., J. Glassberg, J. V. Scott, and A. Kornberg.** 1980. A temperature-sensitive single-stranded DNA-binding protein from *Escherichia coli*. J. Biol. Chem. **255:**2897–2901.
52. **Meyer, R. R., and P. S. Laine.** 1990. The single-stranded DNA-binding protein of *Escherichia coli*. Microbiol. Rev. **54:**342–380.

53. **Meyer, R. R., D. C. Rein, and J. Glassberg.** 1982. The product of the *lexC* gene of *Escherichia coli* is single-stranded DNA-binding protein. J. Bacteriol. **150:**433–435.
53a. **Meyer, R. R., and S. M. Ruben.** Unpublished data.
54. **Meyer, R. R., S. M. Ruben, S. E. VanDenBrink-Webb, P. S. Laine, F. W. Perrino, and D. C. Rein.** 1988. Protein-protein interactions of *Escherichia coli* single-stranded DNA-binding protein, p. 154–162. *In* R. E. Moses and W. C. Summers, (ed.), DNA replication and mutagenesis. American Society for Microbiology, Washington, D.C.
55. **Meyer, R. R., D. W. Voegele, S. M. Ruben, D. C. Rein, and J. M. Trela.** 1982. Influence of single-stranded DNA-binding protein on recA induction in *Escherichia coli*. Mutat. Res. **94:**299–313.
56. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
57. **Neidhardt, F. C., and R. A. vanBogelen.** 1987. Heat shock response, p. 1334–1345. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
57a. **Perrino, F. W., D. C. Rein, and R. R. Meyer.** Unpublished observations.
58. **Phillips, G. J., and T. J. Silvavy.** 1990. Heat shock proteins DnaK and GroEL facilitate export of LacZ hybrid proteins in *E. coli*. Nature (London) **344:**882–884.
59. **Reading, D. S., R. L. Hallberg, and A. M. Myers.** 1989. Characterization of the yeast hsp60 gene coding for a mitochondrial assembly factor. Nature (London) **337:**655–659.
60. **Ruben, S. M., S. E. VanDenBrink-Webb, D. C. Rein, and R. R. Meyer.** 1988. Suppression of the *Escherichia coli ssb-1* mutation by an allele of *groEL*. Proc. Natl. Acad. Sci. USA **85:**3767–3771.
61. **Ruvolo, P. P., K. M. Keating, K. R. Williams, and J. W. Chase.** 1991. Single-stranded DNA binding proteins (SSBs) from prokaryotic transmissible plasmids. Proteins Struct. Funct. Genet. **9:**120–134.
62. **Schlessinger, M. J.** 1990. Heat shock proteins. J. Biol. Chem. **265:**12111–12114.
63. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.
64. **Tessman, E. S., and P. K. Peterson.** 1982. Suppression of the *ssb-1* and *ssb-113* mutations of *Escherichia coli* by a wild-type *rep* gene, NaCl, and glucose. J. Bacteriol. **152:**572–583.
65. **Tilly, K., and C. Georgopoulos.** 1982. Evidence that the two *Escherichia coli groE* morphogenetic gene products interact in vivo. J. Bacteriol. **149:**1082–1088.
66. **Tilly, K., and M. Yarmolinsky.** 1989. Participation of *Escherichia coli* heat shock proteins DnaJ, DnaK, and GrpE in P1 plasmid replication. J. Bacteriol. **171:**6025–6029.
67. **Van Dyk, T. K., A. A. Gatenby, and R. A. LaRossa.** 1989. Demonstration by genetic suppression of interaction of GroE products with many proteins. Nature (London) **342:**451–453.
68. **Viitanen, P. V., G. K. Donaldson, G. H. Lorimer, T. H. Lubbens, and A. A. Gatenby.** 1991. Complex interaction between the chaperonin 60 molecular chaperone and dihydrofolate reductase. Biochemistry **30:**9716–9723.
69. **Wada, M., and H. Itikawa.** 1984. Participation of *Escherichia coli* K-12 *groE* gene products in the synthesis of cellular DNA and RNA. J. Bacteriol. **157:**694–696.
70. **Whittier, R. F., and J. W. Chase.** 1981. DNA repair in *E. coli* strains deficient in single-strand DNA binding protein. Mol. Gen. Genet. **183:**341–347.
71. **Wickner, S. H.** 1990. Three *Escherichia coli* heat shock proteins are required for P1 plasmid DNA replication: formation of an active complex between *E. coli* DnaJ protein and P1 initiator protein. Proc. Natl. Acad. Sci. USA **87:**2690–2694.
72. **Williams, K. R., J. B. Murphy, and J. W. Chase.** 1984. Characterization of the structural and functional defect in the *Escherichia coli* single-stranded DNA binding protein encoded by the *ssb-1* mutant gene. Expression of the *ssb-1* gene under λ $P_L$ regulation. J. Biol. Chem. **259:**11804–11811.
73. **Zeilstra-Ryalls, J., O. Fayet, and C. Georgopoulos.** 1991. The universally conserved GroE (Hsp60) chaperonins. Annu. Rev. Microbiol. **45:**301–325.
74. **Zhou, Y.-N., N. Kusukawa, J. W. Erickson, C. A. Gross, and T. Yura.** 1988. Isolation and characterization of *Escherichia coli* mutants that lack the heat shock sigma factor $\sigma^{32}$. J. Bacteriol. **170:**3640–3649.