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Suppression of the *Escherichia coli ssb-1* mutation by an allele of *groEL*

(DNA replication/temperature-sensitive growth)

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ABSTRACT **A series of spontaneous suppressors to the temperature-sensitive phenotype of the single-stranded DNA-binding protein mutation *ssb-1* were isolated. A genomic library of *Eco*RI fragments from one of these suppressor strains was prepared by using pBR325 as the cloning vector. A 10.0-kilobase class of inserts was identified as carrying the *ssb-1* gene itself. A second class of 8.3-kilobase inserts was shown to contain the *groE* region by (*i*) restriction analysis, (*ii*) Southern hybridization of the 8.3-kilobase insert to *groE*$^+$ DNA, and (*iii*) identification of the gene products by similar migration on polyacrylamide gels. Subcloning demonstrated that an intact mutant *groEL* gene was necessary for suppression and that plasmids carrying the 8.3-kilobase insert could suppress mutants carrying *groES*$^-$ but not *groEL*$^-$ genes for phage λ growth. The suppressor, designated as *groEL411*, was specific for the *ssb-1* allele. In *ssb-1 groEL411* cells, DNA synthesis stopped after a shift to 42.5°C but rapidly recovered within minutes. The data suggest a direct interaction between the single-stranded DNA-binding protein and GroEL proteins in DNA replication.**

It is now well established that single-stranded DNA-binding protein (SSB) plays a key role in DNA metabolism (for reviews, see refs. 1–3). The identification of two temperature-sensitive mutations in the *ssb* gene, *ssb-1* (4) and *ssb-113* (5), were instrumental in demonstrating that SSB is intimately involved in DNA replication, repair, and recombination. Central to its functions, SSB binds preferentially, tightly, and cooperatively to single-stranded DNA (1–3). However, SSB must undoubtedly interact with a variety of proteins. Several protein–protein interactions have been directly demonstrated biochemically, including interactions between SSB and DNA polymerase II (6), between SSB and exonuclease I (7), or between SSB and protein n (8). By using SSB-affinity chromatography, we have demonstrated physical interactions with three *Escherichia coli* proteins, one of which is a folded chromosomal protein (9). Other interactions with Rep protein (10), Rho protein (11), and RecA protein (12) have been suggested.

A powerful genetic approach to the identification of protein–protein interactions involves the isolation of extragenic suppressors. We have isolated a series of independent, spontaneous revertants of *ssb-1*, selected for their ability to synthesize DNA and grow at 42.5°C. In this report we show that the locus for one of these suppressors, *sup411*, lies within the *groE* locus. This locus was originally identified by the inability of mutants to support growth of certain bacteriophages (13) and later shown to code for two proteins, GroEL and GroES (14). The existence of conditionally lethal mutations of *groE* (15) indicates a vital, but as yet undetermined, role for these proteins in bacterial growth. At least one *groES* mutant strain is temperature-sensitive for DNA synthesis (16) and overproduction of wild-type GroE proteins can suppress certain *dnaA* mutations (17, 18). The data reported here indicate that the GroE proteins may be involved in DNA replication through an interaction with SSB.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. All bacterial strains were derivatives of the *E. coli* K12 strain C600, except for HB101 which is a B strain. Relevant markers or strain construction are indicated in the text. Plasmid pSR6 (*ssb*$^+$) has been described (19); other plasmids constructed with pBR325 are described in the text.

Agarose Gel Electrophoresis. Restriction fragments of DNA were separated on 0.8% agarose (Bio-Rad, low molecular weight) at 5 V/cm in Tris borate (0.089 M Tris·HCl/0.089 M boric acid/0.002 M EDTA, pH 8.3). DNA was stained with ethidium bromide (0.5 μg/ml) and visualized at 305 nm. When DNA was to be recovered from the gels for subcloning, low-melting-temperature agarose (SeaPlaque; FMC, Rockland, ME) was used with Tris acetate buffer (0.04 M Tris acetate/0.001 M EDTA, pH 8.3) with buffer recirculation. Ethidium bromide was included in the latter gels and buffer.

Cloning Procedures. A plasmid library was constructed by partial *Eco*RI digestion and cloning in plasmid pBR325 of genomic DNA from strain RM304 [*ssb-1 zjb-1*::Tn*10*; derived from RM121 (19) but carrying the *ssb-1* suppressor]. This DNA was used to transform strain RM121 (*ssb-1*) as described by Hanahan (20); colonies were selected that were ampicillin resistant at 42.5°C and that were chloramphenicol sensitive. Plasmid DNA was isolated from positive clones and used to transform RM121 a second time to confirm suppression of *ssb-1*. The 8.3-kilobase (kb) fragment from pSR411, carrying the suppressor, was subcloned in pBR325 after digesting with *Eco*RI plus *Ava* I, *Pst* I, or *Bam*HI and separating the fragments on 0.9% agarose gels. The appropriate fragments were excised, ligated *in situ* with appropriately digested pBR325, and used to transform strain HB101 (*recA*; ref. 21).

Hybridization. The purified 8.3-kb fragment was nick-translated by using *E. coli* DNA polymerase I and [^{32}P]dTTP and used as a probe for hybridization to agarose gel-separated *Eco*RI-restricted DNA fragments as described by Southern (22).

"Maxicell" Labeling. Recombinant plasmids were transformed into strain RB113 (*uvrA recA*; ref. 23), and the transformed cells were grown in M9 medium supplemented

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Abbreviation: SSB, single-stranded DNA-binding protein.

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with amino acids at 50 μg/ml except methionine and cysteine. After UV-irradiation at 1 J/m^2 and cycloserine treatment, cells were labeled with [^{35}S]methionine at 5 μCi/ml (1100 Ci/mmol; 1 Ci = 37 GBq) for 1 hr as described by Sancar *et al.* (24). After labeling, cell extracts were prepared, and 50-μl aliquots were electrophoresed and fluorographed as described (19).

Plating Efficiency. Cells, grown overnight at 32°C, were diluted 1:100 into L broth and grown to an A_{595} of 0.5. Appropriate dilutions on L-broth plates were incubated overnight at 32 or 42.5°C and colonies were counted.

RESULTS

Isolation of a Suppressor to *ssb-1*. Fifty-one independent spontaneously arising mutants suppressing the temperature-sensitive phenotype expressed by *ssb-1* in strain RM121 (19) were isolated at 42.5°C. The spontaneous reversion frequency was determined to be 10^{-5}. One of these strains, RM304, was chosen for further analysis. The suppressor locus (designated *sup411*) could be cotransduced by phage P1, but at a low frequency. This indicated that *sup411* did not lie within the *ssb-1* allele and simultaneously provided a region of the chromosome to search for its identity.

Cloning of *sup411*. A genomic library of strain RM304 was prepared from partial digestion of chromosomal DNA with *Eco*RI and cloned into pBR325. These plasmids were used to transform RM121 (*ssb-1*) and selected for suppression of the temperature-sensitive phenotype, expressed by *ssb-1*, by growth at 42.5°C. Twenty-two positive clones were isolated, and plasmid DNA was prepared. Analysis of the *Eco*RI restriction digests of plasmid DNA indicated that there were two distinct classes of RM304 fragments that were responsible for suppression: an 8.3-kb class and a 10.0-kb class. Both classes were found to have some plasmids that contained other fragments in addition to the 8.3-kb or 10.0-kb fragment. This was not unexpected, as only a partial *Eco*RI digestion was used in the original cloning.

Analysis of Plasmid-Encoded Proteins. The plasmid proteins were identified by maxicell labeling (24). Recombinant plasmids from each of the two *Eco*RI size classes were used to transform strain RB113 (*recA uvrA*). Fig. 1 is an autoradiograph of these extracts. One of these plasmids (pSR438), carrying the 10.0-kb insert, codes for a prominent protein of M_r ≈19,000, which is close to the molecular weight of SSB (M_r, 18,873). However, *ssb* has been shown to be present on

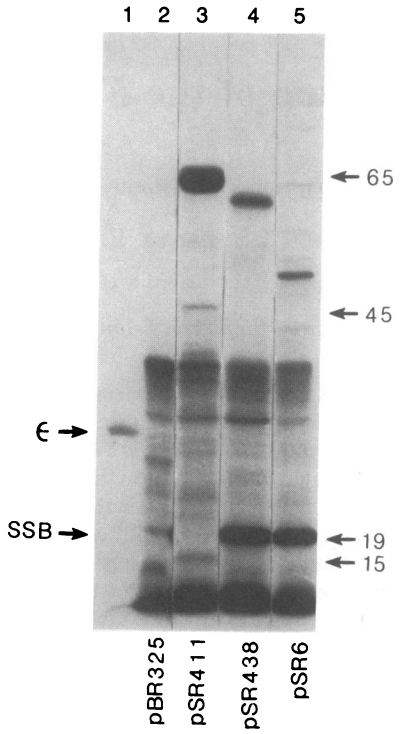

FIG. 1. Extracts of maxicell-labeled cells containing representative recombinant plasmids. Maxicell extracts with [^{35}S]methionine were electrophoresed and fluorographed. Plasmid pSR6, coding for SSB (M_r 19,000, lane 5) and the purified ε subunit (M_r 25,000) of DNA polymerase III holoenzyme (lane 1) were used as standards. An extract of pSR438, representing one of the 10.0-kb inserts, is shown in lane 4; pSR411, representing the 8.3-kb insert class, is shown in lane 3. A vector pBR325 extract was used as a control to distinguish vector-encoded proteins (lane 2). The 10.0-kb fragment codes for SSB-1 protein; the 8.3-kb fragment codes for proteins of M_rs 15,000, 45,000, and 65,000.

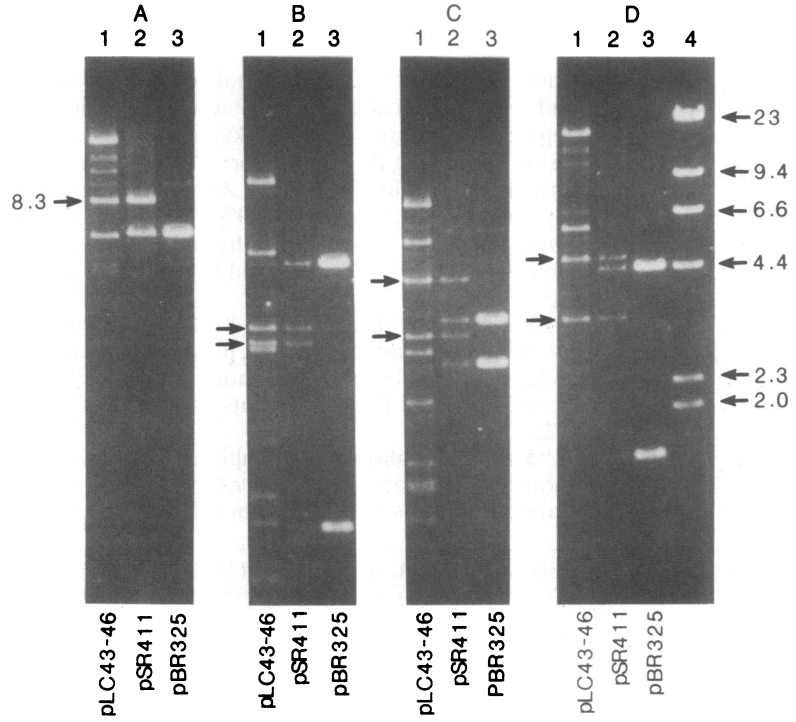

FIG. 2. Comparison of restriction fragments of pSR411 and the Clarke–Carbon plasmid pLC43-46 (*groE*$^+$). Agarose gel electrophoresis separation of pLC43-46 (lanes 1), pSR411 (lanes 2), and vector pBR325 DNA (lanes 3), digested with various restriction enzymes. *A*, *Eco*RI; *B*, *Eco*RI and *Bam*HI; *C*, *Eco*RI and *Ava* I; *D*, *Eco*RI and *Pst* I. λ DNA digested with *Hin*dIII (*D*, lane 4) was used as molecular size standards. The similar fragments of pSR411 and pLC43-46 are marked in each panel by arrows.

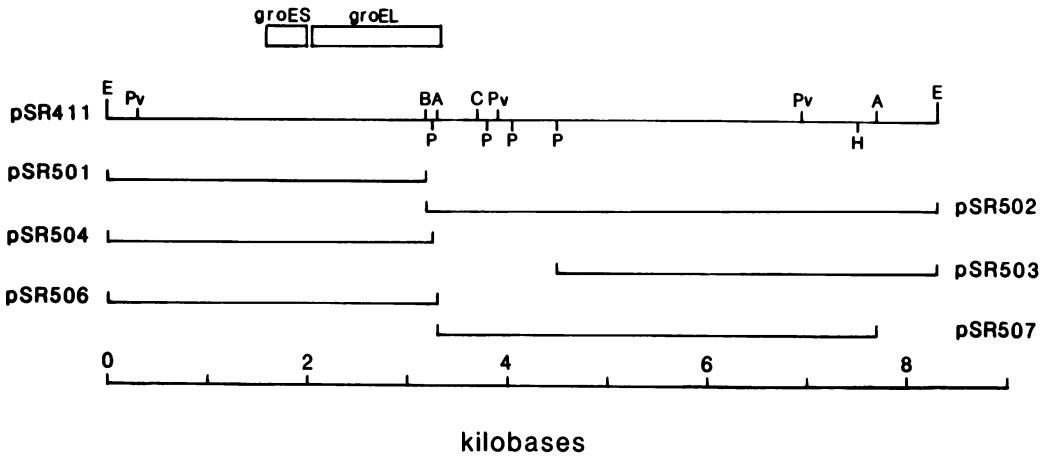

FIG. 3. Restriction map of the 8.3-kb fragment. Restriction endonucleases were as follows: A, *Ava* I; B, *Bam*HI; C, *Cla* I; E, *Eco*RI; H, *Hin*dIII; P, *Pst* I; Pv, *Pvu* I. The fragments subcloned are indicated in the figure with the designated plasmid name.

a 6.6-kb *Eco*RI fragment (25). The similar migration and immunoblot analyses of the proteins and similar restriction analysis of the DNA (data not shown) confirm that the M_r 19,000 protein band, indeed, represents SSB-1, the protein encoded by *ssb-1*. Therefore, the differences in the *Eco*RI fragment size simply reflects strain differences. It is known that overproduction of SSB-1 protein suppresses the temperature-sensitive phenotype expressed by *ssb-1* (26).

Plasmid pSR411, containing the 8.3-kb fragment, codes for three proteins of M_r values 15,000, 45,000, and 65,000. Because *sup411* cotranduces with *ssb-1*, we searched the *E. coli* genetic map within 2 min on either side of *ssb* (at 92.1 min) for genes coding for proteins of these sizes. None of the known replication proteins in this region have these molecular weights. However, the *groE* locus at 94.0–94.2 min (17, 18) codes for two proteins, GroEL of M_r 65,000–68,000 and GroES of M_r 15,000 (14).

Comparison of the 8.3-kb Fragment to the Wild-Type *groE* Genes. Plasmid pLC43-46 of the Clarke–Carbon *E. coli* library (27) cloned in ColE1 has been identified as containing the wild-type *groE* genes (28). A comparison of the restriction maps of pSR411 and pLC43-46 is shown in Fig. 2. These plasmids were first digested with *Eco*RI to generate the 8.3-kb fragment and then with other restriction enzymes. Ignoring the fragments resulting from the pBR325 and ColE1 vectors, it is apparent that the remaining restriction fragments of pSR411 comigrate with fragments of pLC43-46. A restriction map of the 8.3-kb fragment is shown in Fig. 3 and is consistent with partial maps of the *groE* region (17, 18). As a further confirmation, the 8.3-kb fragment was hybridized to the digested plasmid pLC43-46 (Fig. 4). The probe hybridized only to itself (lane 3) and to a fragment of similar mobility from pLC43-46 (lane 2). These data, along with the results of the maxicell labeling and restriction analysis, have identified the 8.3-kb *Eco*RI fragment, coding for GroEL and GroES proteins, as carrying the *sup411* suppressor allele of the *ssb-1* gene temperature-sensitive phenotype.

The *groEL* Gene Is Required for Suppression of *ssb-1*. The 8.3-kb fragment could theoretically code for several proteins, and our maxicell labeling (Fig. 1) indicates at least three labeled proteins of M_r values 65,000, 45,000, and 15,000 from pSR411. To test whether *groEL* and/or *groES* are required for suppression, these genes were inactivated by generating deletions. Three restriction enzymes were used, and the digested 8.3-kb fragments were subcloned in pBR325. These subclones are indicated in Fig. 3. None of the fragments could suppress *ssb-1*. Maxicell labeling (Fig. 5) indicated that all contained either a truncated GroEL protein (pSR501 and pSR506) or lacked a GroEL protein (pSR502, pSR503, and pSR507). Two plasmids (pSR501 and pSR504), however, contained an intact *groES* gene, and two plasmids (pSR507 and pSR502) showed increased expression of the M_r 45,000 protein. These data indicate that an intact *groEL* gene is required for suppression.

As a final test, pSR411 was transformed into strains CG714 (*groEL140*) and CG712 (*groES30*) (14), and the resultant strains were tested for their ability to support phage λ growth. Plasmid pSR411 complemented the *groES30* mutation, but not the *groEL140* mutation, providing further evidence that the *sup411* suppressor of *ssb-1* resides in the *groEL* gene. Consequently, we have redesignated *sup411* as *groEL411*.

Specificity of Suppression. To test the allelic specificity of *groEL411*, strain RM139 (*ssb-113*) was transformed with pSR411. No suppression of the temperature sensitivity of the *ssb-113* allele occurred (Table 1), indicating that suppression is specific for the *ssb-1* mutation. In another set of experiments, plasmids carrying the wild-type $groEL^+$ and $groES^+$ genes were used to transform RM121 (*ssb-1*) and RM139 (*ssb-113*). The wild-type $groEL^+/groES^+$ were unable to suppress the temperature sensitivity of either *ssb* mutation. These results show that suppression is specific for the *ssb-1* allele and is dependent upon a difference between the wild-type and suppressor *groEL411* allele.

Kinetics of DNA Synthesis After Temperature Shift. DNA synthesis ceases rapidly after temperature shift *in vivo* in *ssb-1* cells or *in vitro* with mutant protein (4, 29). Thus, it was of interest to examine the kinetics of synthesis in the

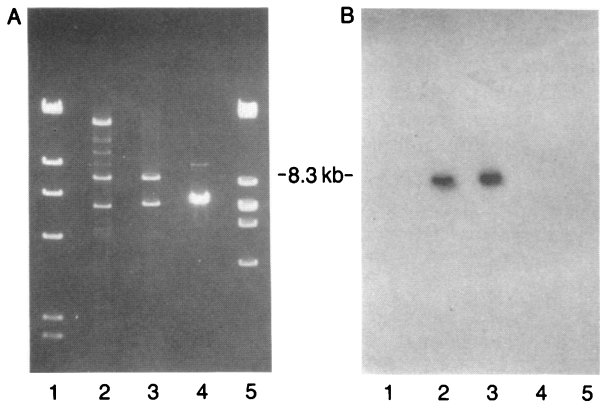

FIG. 4. Southern hybridization of the 8.3-kb fragment to pLC43-46. A ^{32}P-labeled 8.3-kb probe was prepared from pSR411 and hybridized to *Eco*RI-digested and agarose gel-separated pSR411 and pLC43-46. (*A*) Agarose gel. (*B*) Autoradiograph of gel in *A*. Lanes: 1, λ DNA digested with *Hin*dIII; 2, pLC43-46; 3, pSR411; 4, pBR325; 5, λ DNA digested with *Eco*RI. The probe hybridized to itself (*B*, lane 3) and to the 8.3-kb $groE^+$ fragment of pLC43-46 (*B*, lane 2).

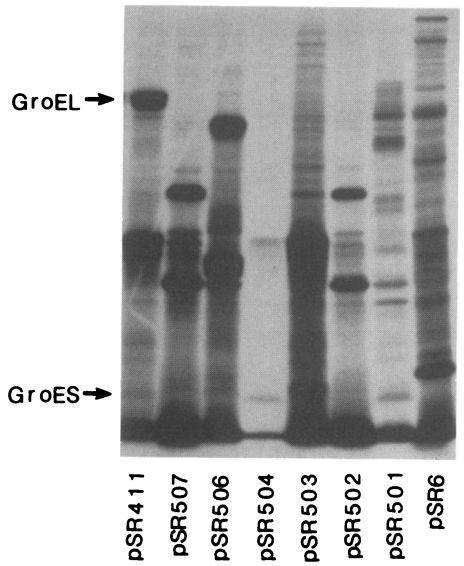

FIG. 5. Maxicell-labeled extracts from subcloned fragments of the 8.3-kb insert. Plasmid pSR411 was digested with several nucleases and subcloned in pBR325. Maxicell extracts were prepared and fluorographed. Plasmids pSR501 contained an *Eco*RI–*Bam*HI insert; pSR502 contained a *Bam*HI–*Eco*RI insert; pSR503 contained a *Pst* I–*Eco*RI insert; pSR504 contained an *Eco*RI–*Pst* I insert; pSR506 contained an *Eco*RI–*Ava* I insert; pSR507 contained an *Ava* I–*Eco*RI insert. Extracts of pSR6 coding for SSB and pSR411 coding for GroEL and GroES proteins are shown as controls.

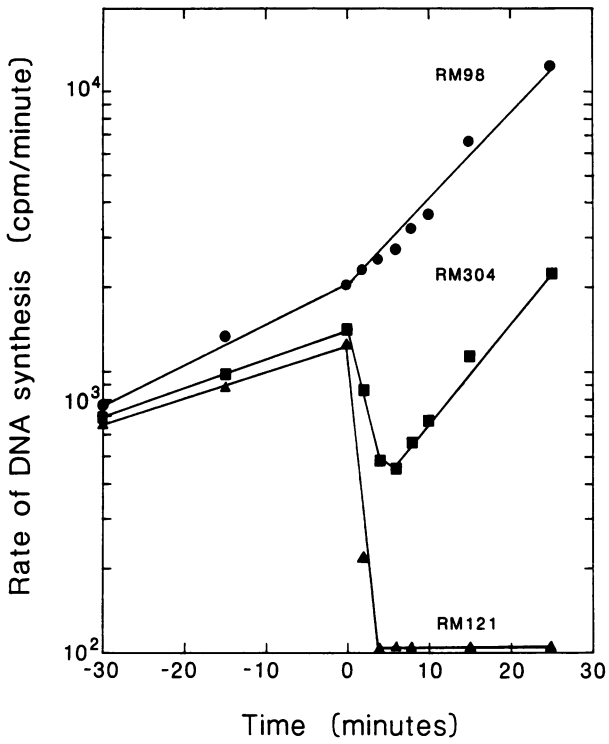

FIG. 6. Rate of DNA synthesis after temperature shift. Cells grown in L broth to an A_{595} of 0.2 at 32°C were pipetted into flasks prewarmed at 42.5°C. At the indicated times, 0.5-ml aliquots were pulse-labeled with 3 μCi of [^{3}H]thymidine for 3 min (42.5°C) or 5 min (32°C). Reactions were stopped with 2 ml of ice-cold 10% (wt/vol) trichloroacetic acid. At the zero-time point, labeling was carried out at 32°C. ●, RM98 (*ssb*$^+$); ▲, RM121 (*ssb-1*); and ■, RM304 (*ssb-1 groEL411*).

suppressor strain, as it could provide some insight into the mechanism of suppression. Fig. 6 shows that *ssb*$^+$ cells continue synthesis after temperature shift, whereas *ssb-1* cells rapidly stop synthesis and do not recover. The effect on the strain containing *ssb-1* and *groEL411* was surprising. Within 1 min after shift, the rate of synthesis dropped in parallel with the *ssb-1* cells, but within 5 min the rate of synthesis was again increasing logarithymically. Moreover, the slope of the line for this strain was identical to that of the *ssb*$^+$ cells at 42.5°C.

DISCUSSION

A spontaneously arising suppressor of the single-stranded DNA-binding protein mutation *ssb-1* has been isolated and shown to lie within the *groE* locus. The data strongly indicate that it is a mutation of *groEL*, as inactivation of this gene by subcloning results in loss of suppressor activity. Furthermore, a plasmid carrying the suppressor gene is able to complement a chromosomal mutation for λ growth in *groES30* cells, but not in *groEL140* cells. It should be noted, however, that the data do not exclude the possibility of a second essential mutation in *groES* for suppression. Overproduction of wild-type GroE$^+$ proteins, which are capable of suppressing certain *dnaA* mutations (17, 18), cannot suppress *ssb-1*. The suppression is specific for the *ssb-1* allele, as it does not suppress *ssb-113*, suggesting that the suppressor is not simply replacing mutant SSB in replication.

Table 1. Plating efficiency of strains at 32°C and 42.5°C

Strain and marker(s)	Colony-forming units/ml, no. × 10^{-8} 32°C	42.5°C	Plating efficiency at 42.5°C, %
RM98 (*ssb*$^+$)	3.29	3.16	96.0
RM121 (*ssb-1*)	1.69	0.000015	0.0088
RM304 (*ssb-1 groEL411*)	2.50	2.57	102.8
RM121 (*ssb-1*)/pSR411 (*groEL411*)	0.84	0.75	89.3
RM139 (*ssb-113*)	1.62	0.000345	0.021
RM139 (*ssb-113*)/pSR411 (*groEL411*)	0.53	0.000185	0.035

The role of GroE proteins in the morphogenesis of phages λ, T4, T5, and others is well established (13, 30). The involvement of *groE* in DNA metabolism has been suggested by the observations that the levels of GroE proteins increase with shorter generation times (and hence increased number of replication forks) (31) and that some *groE* mutants are conditionally lethal (15). The suppression of certain *dnaA* mutations by GroE$^+$ proteins (17, 18) argues for a role, either direct or indirect, in initiation of chromosomal replication at *oriC* where *dnaA* is required (32). At the initiation stage, several replication proteins form a multiprotein complex (33). With their known ability to organize various phage proteins in morphogenesis, the GroE proteins may be involved in the organization of the initiation complex *in vivo*. While this could provide a role for GroE proteins at the level of chromosomal initiation, it should be noted that *ssb-1* has a fast-stop phenotype at 42°C (29) and, thus, is involved in chain elongation. The functional structure of SSB is probably a tetramer (34). Williams *et al.* (35) have examined the effects of SSB-1 concentration and temperature on DNA binding. At concentrations at which SSB is present in the cell, SSB-1 tetramers tend to dissociate to monomers. Surprisingly, at elevated temperatures the SSB-1 tetramers are stable, although the monomers appear to be nonfunctional. Consequently these monomers may no longer be capable of DNA binding or functional tetramer formation. This offers some interesting speculations. Overproduction of SSB-1 is known to overcome many of the defects of the *ssb-1* mutation (26),

probably by shifting the equilibrium toward functional SSB-1 tetramers (35). Although *groEL411* mutant cells have a somewhat elevated level of SSB-1 protein (S.M.R., P. S. Laine, S.E.V.D.B.-W., D.C.R., and R.R.M., unpublished results), these levels are probably not sufficient to suppress the temperature-sensitive phenotype. It seems more likely that GroEL411 protein interacts directly with SSB-1 either to suppress dissociation of SSB-1 tetramers or to actively reorganize SSB-1 monomers back into tetramers. This speculation would be consistent with the known ability of GroE proteins to organize phage proteins during morphogenesis. Since SSB-1 can be boiled and regain full activity after cooling (29), the inactive monomers are not irreversibly denatured at restrictive temperatures and would be available for recycling. The decrease in DNA synthesis, followed by rapid recovery in strain RM304 (Fig. 6), would be consistent with this mechanism.

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