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Error-Prone DNA Polymerase IV Is Regulated by the Heat Shock Chaperone GroE in *Escherichia coli*

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An insertion in the promoter of the operon that encodes the molecular chaperone GroE was isolated as an antimutator for stationary-phase or adaptive mutation. The *groE* operon consists of two genes, *groES* and *groEL*; point mutations in either gene conferred the same phenotype, reducing Lac$^+$ adaptive mutation 10- to 20-fold. *groE* mutant strains had 1/10 the amount of error-prone DNA polymerase IV (Pol IV). In *recG*$^+$ strains, the reduction in Pol IV was sufficient to account for their low rate of adaptive mutation, but in *recG* mutant strains, a deficiency of GroE had some additional effect on adaptive mutation. Pol IV is induced as part of the SOS response, but the effect of GroE on Pol IV was independent of LexA. We were unable to show that GroE interacts directly with Pol IV, suggesting that GroE may act indirectly. Together with previous results, these findings indicate that Pol IV is a component of several cellular stress responses.

Members of the recently discovered Y family of DNA polymerases are highly error prone and poorly processive (22, 63). *Escherichia coli* has two members of this family, DNA polymerase IV (Pol IV), encoded by the *dinB* gene (59), and DNA Pol V, encoded by the *umuD* and *umuC* genes (48, 54, 56). Both of these polymerases are induced by DNA damage as part of the SOS response (2, 8, 28). Pol V is able to replicate past a variety of DNA lesions and is rightly called a lesion bypass polymerase; however, Pol IV has only a limited ability to replicate damaged DNA (30, 44, 55) and its true role in the cell remains speculative.

When populations of microorganisms are placed under certain nonlethal selective conditions, mutations that relieve the selective pressure arise, a process called adaptive mutation (7, 16). Most of the research on adaptive mutation has focused on *E. coli* strain FC40 (6). FC40 cannot utilize lactose because of a frameshift mutation that affects the *lacZ* gene; when FC40 cells are plated with lactose as the sole carbon and energy source, the Lac$^-$ population remains stable but Lac$^+$ revertants arise at a constant rate for about a week (6). Unlike growth-dependent mutations, adaptive mutations in FC40 require functions for recombination (6, 17, 20, 23, 24). In addition, the production of Lac$^+$ adaptive mutations in FC40 is enhanced when the mutational target is on a conjugal plasmid and conjugal functions are expressed (19, 21, 46). The last result probably reflects the fact that DNA nicking at the conjugal origin stimulates the mutational process (50).

In FC40, loss of Pol IV reduces the rate of adaptive mutation three- to fivefold (18). Recently we reported that Pol IV is induced late in stationary phase under the positive control of RpoS, the stationary-phase sigma factor. After induction, high levels of Pol IV are maintained in the starving cells for at least 3 days, but only if the cells are *rpoS*$^+$ (34). Other researchers have shown that the *dinB* gene is transcribed in a 5-day-old culture (63). In an *rpoS* mutant strain, the rate of Lac$^+$ adaptive mutation is reduced 5- to 10-fold (34, 39). About half of this reduction is due to the decreased amount of Pol IV in the *rpoS* mutant strain, and the rest is due to some other effect that RpoS has on adaptive mutation (34).

The results summarized above indicate that Pol IV is induced as part of two stress responses: the SOS response to DNA damage, under the control of the LexA repressor, and the starvation response, positively regulated by RpoS. Both of these responses are also induced under a variety of other conditions. The SOS response is induced at the end of growth in liquid rich medium (11) and in aging colonies on solid rich medium (52). RpoS is responsive to stresses in addition to starvation that tend to inhibit active cell growth (25). Thus, cells respond to a variety of difficult situations by increasing their levels of Pol IV, which would tend to increase the error rate of DNA synthesis during repair or replication. Here we report that levels of Pol IV are also positively affected by the heat shock-induced molecular chaperone GroE, bringing to three the number of stress responses that influence Pol IV.

MATERIALS AND METHODS

Bacterial strains. The strains used are listed in Table 1. Genetic manipulations were performed using standard techniques (43). The original isolation of the *groES*::Cm mutant was as previously described for the *rpoS*::Cm mutant (34). To make *groEL100* and *groES30* derivatives, parental strains were transduced to tetracycline resistance (Tet^r) with bacteriophage P1$_{vir}$ lysates of strains GW7503 (= AB1157 *groEL100 purA*::Tn*10*) and GW7504 (= AB1157 *groES30 purA*::Tn*10*) (9). Tet^r, adenine-requiring (Ade$^-$) candidates were tested for resistance to λ_{vir} bacteriophage to identify sensitive (*groE*$^+$) and resistant (*groE*$^{[minus]}$) isogenic pairs. The *rpoS*::Cm and *groES*::Cm alleles were transferred by P1$_{vir}$ transduction, with selection for chloramphenicol resistance (Cm^r). Tet^s Arg$^+$ derivatives of TE8197 and TE8222 were constructed by transducing them to arginine prototrophy with a P1$_{vir}$ lysate of FC36 and then screening Arg$^+$ isolates for sensitivity to tetracycline. Strains that have *dinB* deleted on both the chromosome and the episome were constructed as previously described (34) except that the nonpolar *dinB*::Zeo allele was used (5) and selection was for resistance to zeomycin (Zeo^r). F′ Φ(*lacI33-lacZ*) Pro$^+$ was mated into F$^-$ Pro$^-$ strains by conjugation with a Met$^-$/F′ Φ(*lacI33-lacZ*) Pro$^+$ donor, with selection for proline and methionine prototrophy.

Mutation, growth, and viability assays. Media and experimental protocols were as previously described (6, 15, 20). As required, minimal medium was

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TABLE 1. *E. coli* strains and plasmids used in this study

Strain(s)	Relevant phenotype or genotype	No. of copies of *dinB*$^+$	Reference
FC29	*ara* Δ(*gpt-lac*)*5 thi*/F′ Δ(*lacIZ*) Pro$^+$	2	6
FC36	*ara* Δ(*gpt-lac*)*5 thi* Rif^r	1	6
FC40	FC36/F′ Φ(*lacI33-lacZ*) Pro$^+$	2	6
FC526	FC40 Δ*recG263*::Kn	2	20
FC1230	FC40/pYG768	Many	This study and reference 29
FC1240	FC722 Δ*dinB*::Kn on chromosome and episome	None	34
PF2244	TE8197[a] Arg$^+$ Tet^s	1	This study and reference 26
PF2245	TE8222[b] Arg$^+$ Tet^s	1	This study and reference 26
PF2252	WAM106[c] P_L-*lacZ*	1	61
PF2253	WAM106[c] P*groE-lacZ*	1	61
PF2258	PF2252 *rpoS*::Cm	1	This study and reference 34
PF2259	PF2253 *rpoS*::Cm	1	This study and reference 34
PFG60, PFG64[d]	FC40 *groEL*$^+$ *purA*::Tn*10*	2	This study
PFG61	FC40 *groEL100 purA*::Tn*10*	2	This study and reference 66
PFG62, PFG66[d]	FC526 *groEL*$^+$ *purA*::Tn*10*	2	This study
PFG63	FC526 *groEL100 purA*::Tn*10*	2	This study
PFG65	FC40 *groES30 purA*::Tn*10*	2	This study and reference 33
PFG67	FC526 *groES30 purA*::Tn*10*	2	This study
PFG250	FC40 *groE*$^+$ *purA*::Tn*10* Δ*dinB*::Zeo on chromosome and episome	None	This study and reference 5
PFG251	FC40 *groEL100 purA*::Tn*10* Δ*dinB*::Zeo on chromosome and episome	None	This study
PFG305	FC526 *groE*$^+$ *purA*::Tn*10* Δ*dinB*::Zeo on chromosome and episome	None	This study
PFG307	FC526 *groEL100 purA*::Tn*10* Δ*dinB*::Zeo on chromosome and episome	None	This study
PFG308	FC36 *sulA-lacZ groEL*$^+$ *purA*::Tn*10*	1	This study
PFG309	FC36 *sulA-lacZ groEL100 purA*::Tn*10*	1	This study
PFG332	PFG308 *recG263*::Kn	1	This study
PFG333	PFG309 *recG263*::Kn	1	This study
PFG324	FC36 *sulA11 groEL*$^+$ *purA*::Tn*10* *lexA71*::Kn	1	This study and reference 34
PFG325	FC36 *sulA11 groEL100 purA*::Tn*10* *lexA71*::Kn	1	This study and reference 34
PFG326	FC36 *sulA11 groEL*$^+$ *purA*::Tn*10*	1	This study and reference 34
PFG327	FC36 *sulA11 groEL100 purA*::Tn*10*	1	This study and reference 34
PFG328	PFG324/F′ Φ(*lacI33-lacZ*) Pro$^+$	2	This study
PFG329	PFG325/F′ Φ(*lac/33-lacZ*) Pro$^+$	2	This study
PFG330	PFG326/F′ Φ(*lacI33-lacZ*) Pro$^+$	2	This study
PFG331	PFG327/F′ Φ(*lacI33-lacZ*) Pro$^+$	2	This study
PFG334	GJ2770[e] *groEL*$^+$ *purA*::Tn*10*	1	This study
PFG335	GJ2770[e] *groEL100 purA*::Tn*10*	1	This study
PFG336	PFG334 *rpoS*::*Cm*	1	This study
PFG340	PF2244 *groEL*$^+$ *purA*::Tn*10*	1	This study
PFG341	PF2244 *groEL*100 *purA*::Tn*10*	1	This study
PFG342	PF2245 *groEL*$^+$ *purA*::Tn*10*	1	This study
PFG343	PF2245 *groEL*100 *purA*::Tn*10*	1	This study
PFG321	FC40 *groES*::Cm	2	This study
PFG351	FC526 *groES*::Cm	2	This study

[a] TE8197 is MG1655 Δ(*lacIZ*) *argA*::Tn*10 trpDC700*::*putPA1303*::Kn^r-*rpoS-lac* (protein fusion) (26). MG1655 is considered to be a wild-type strain (4).
[b] TE8222 is MG1655 Δ(*lacIZ*) *argA*::Tn*10 trpDC700*::*putPA1303*::Kn^r-*rpoS-lac* (operon fusion) (26).
[c] WAM106 is F$^-$ *araD139* Δ(*argF-lac*)*U169* Δ(*his-gnd*) *thi rpsL150 flbB5301 relA1 deoC1 rbsR* (57).
[d] Both strains were constructed by transduction; the donor for the first was GW7503 (=AB1157 *groEL100 purA*::Tn*10*), and the donor for the second was GW7504 (=AB1157 *groES30 purA*::Tn*10*) (9). Tet^r Ade$^-$ λ_{vir}-sensitive transductants were isolated.
[e] GJ2770 is MC4100 λ [*katE*::*lac*(Kan)] (47). MC4100 is Δ(*argF-lac*)*U169 rpsL150 relA1 araD139 flbB5301 deoC1 ptsF25.*

supplemented with 100 μg of adenine or histidine per ml, 50 μg of tryptophan per ml, 10 μg of tetracycline per ml, 15 μg of kanamycin per ml, or 10 μg of chloramphenicol per ml. Rich medium was supplemented with 100 μg of adenine per ml, 20 μg of tetracycline per ml, 30 μg of kanamycin per ml, 10 μg of chloramphenicol per ml, or 25 μg of zeomycin per ml.

For adaptive-mutation experiments, cells were grown to saturation at 32°C in 1% glycerol–M9 minimal medium (plus adenine when required) (43). Approximately 10^7 cells from each culture were spread on each quadrant of a 1% lactose–M9 minimum medium plate (plus adenine when required), or an appropriate number of cells (10^6 to 10^8) was added to approximately 10^9 FC29 scavenger cells and spread on a 1% lactose–M9 minimum medium plate. Plates were incubated for 5 days at 37°C, and newly arising Lac$^+$ colonies were counted each day. To measure viability, plugs were removed from between the Lac$^+$ colonies and the number of viable cells was determined each day by plating appropriate dilutions on Luria-Bertani plates plus chloramphenicol or tetracycline plus adenine, on which FC29 cannot grow (6). Mutation to Lac$^+$ is given either as the

mean number of Lac$^+$ colonies appearing each day from days 3 to 5 or as the mean number of Lac$^+$ colonies accumulating each day divided by the number of Lac$^-$ cells present 2 days earlier. Statistical calculations were as given in references 49 and 65.

β-Galactosidase assays. Saturated cultures were diluted 1:1,000 into 1% glycerol–M9 minimal medium supplemented with the additive required for each strain and allowed to reach saturation at 37°C. Appropriate dilutions of the cultures were assayed for optical density at 600 nm and β-galactosidase production as described previously (43).

Molecular techniques. Standard molecular biology techniques were used (1). For immunoblots, cells were grown in 1% glycerol–M9 minimal medium plus appropriate additives at 37°C to saturation, and then incubation was continued for 16 to 24 h; this procedure was previously found to result in maximum levels of Pol IV (34). Cells were harvested and boiled in sample loading buffer, and the total protein was measured by Bradford assays (Bio-Rad Laboratories). Samples containing 40 μg of total protein were subjected to electrophoresis in a sodium dodecyl sulfate–12% polyacrylamide gel and then electro-transferred to Immobilon-P membranes (pore size, 0.45 μm; Millipore Co.). Pol IV was visualized with rabbit anti-Pol IV polyclonal antiserum (obtained from H. Ohmori) clarified with acetone powder made from a Δ(*dinB*) strain, followed by reaction with alkaline phosphatase-conjugated goat anti-rabbit secondary antibody, and developed using the Western-light chemiluminescence reagent (Applied Biosystems). Bands were quantitated using ImageJ software (W. Rasband, National Institutes of Health). Coimmunoprecipitations were done with monoclonal antibody to GroEL (Stressgen Biotechnologies Corp.) and protein A or protein G immunoprecipitation kits (Roche Diagnostics Corp). For metal affinity purification, Talon cobalt-resin columns (BD Biosciences Clontech) were used. Yeast two-hybrid analysis was performed with Matchmaker T System 3 (BD Biosciences Clontech).

RESULTS

An insertion in the *groES/groEL* operon reduces adaptive mutation to Lac$^+$. The rate of adaptive mutation to Lac$^+$ in *E. coli* strain FC40 is elevated 10- to 100-fold in *recG* mutant strains (20, 24). This increase is substantially, or entirely, due to elevated levels of Pol IV in *recG* mutant strains (34). To find mutations in genes that regulate the amount or activity of Pol IV, we mutated a *recG* mutant strain with a transposon conveying chloramphenicol resistance, mini-Tn*10*d Cm (31), and screened Cm^r isolates for reduction in adaptive mutation to Lac$^+$ (34). One such mutant was an insertion in the *groES/groEL* operon. *groES* and *groEL* are essential genes that encode the two subunits of the heat shock-inducible molecular chaperone GroE. The mini-Tn*10*d Cm inserted between the −10 and −35 regions of the heat shock (σ^{32}-dependent) promoter, leaving intact the proposed vegetative (σ^{70}-dependent) promoter (see reference 67 for a description of the promoters). Like *E. coli* strains with other mutations in *groES* or *groEL* (58), *groES::*Cm mutant strains are temperature sensitive for viability (at 43°C) and resistant to bacteriophage lambda (data not shown). As shown in Fig. 1, the *groES::*Cm allele reduced Lac$^+$ adaptive mutation in both *recG*$^+$ and *recG* mutant strains. The effect of the *groES::*Cm allele was stronger in the *recG* mutant strain than in the *recG*$^+$ strain.

Point mutations in *groES and groEL* also reduce adaptive mutation to Lac$^+$. Because our *groES*::Cm mutation is unique, we sought to confirm its effects with previously isolated and well-characterized *groE* mutant alleles. The *groES30* allele has two mutations, a G-to-A change in the Shine-Dalgarno sequence of the gene and an A31V mutation in the coding sequence (33). The *groEL100* allele has an S201F mutation that affects the ability of GroEL to bind to GroES and to bind and release substrate proteins (3, 66). Both mutant alleles make cells resistant to bacteriophage, but only the *groEL100* allele makes cells temperature sensitive (58). As shown in Fig. 1, both of these mutant alleles reduced the level of adaptive mutation. As with the *groES::*Cm allele, the reduction in adaptive mutation was greater in the *recG* mutant strain.

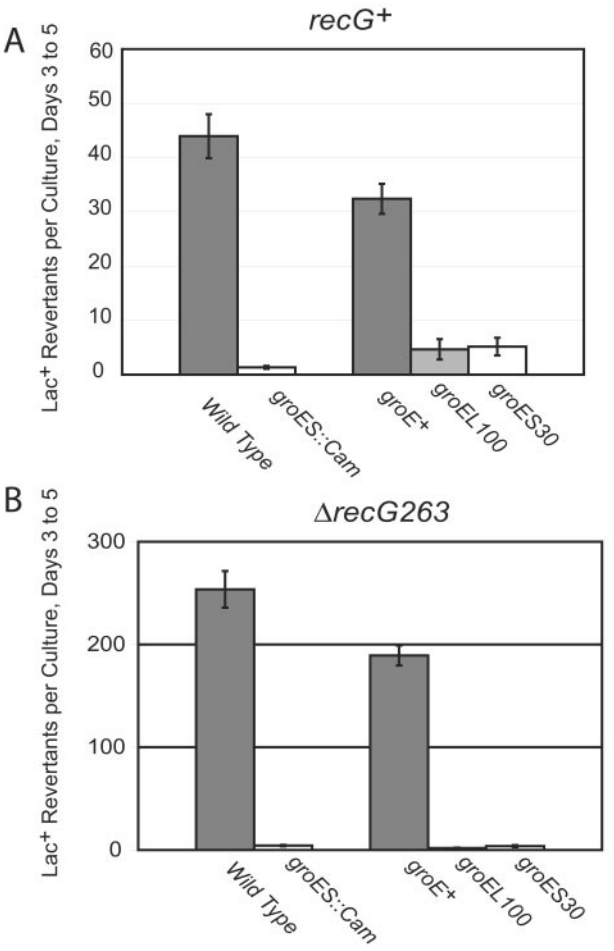

FIG. 1. Mutant alleles of *groE* decrease adaptive mutation in *E. coli*. The accumulation of Lac$^+$ revertants during incubation on lactose-minimal medium. Four to six independent cultures were plated for each strain; data are the mean numbers of Lac$^+$ colonies appearing each day from days 3 to 5 ± standard errors of the means (SEM). The results of four experiments are shown. (A) Wild type = FC40; *groES*::Cm = PFG361; *groE*$^+$ = PFG60 and PFG64; *groEL100* = PFG61; *groES30* = PFG65. (B) Wild type = FC526; *groES*::Cm = PFG351; *groE*$^+$ = PFG62 and PFG66; *groEL100* = G63; *groES30* = PFG67. PFG60 through PFG67 are also *purA*::Tn*10*.

The reduction in adaptive mutation due to GroE deficiency is not due to loss of viability. The results in Fig. 1 were obtained with our semiquantitative assay (20). To obtain quantitative results and to correct for any loss of viability during the experiments, we did large-scale experiments monitoring the number of viable Lac$^-$ cells on the lactose plates every day (6). These results are shown in Fig. 2, which compares *groEL100* and *groE*$^+$ in both *recG*$^+$ and *recG* mutant backgrounds. The large-scale experiments confirmed the previous results: after correction for viable-cell number, GroE deficiency reduced the rate of adaptive mutation to Lac$^+$ about 10-fold in *recG*$^+$ cells and about 20-fold in *recG* mutant cells.

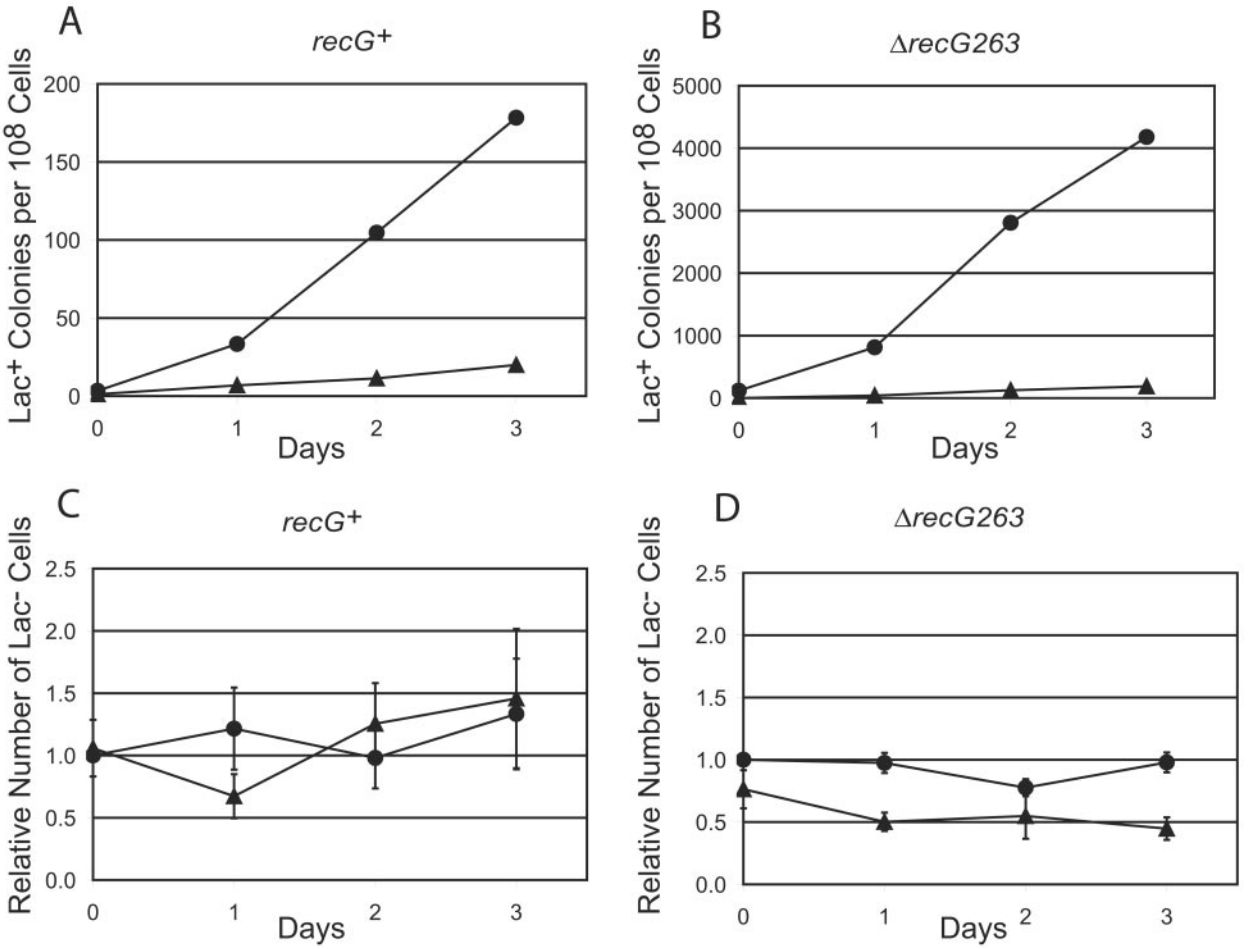

FIG. 2. The reduction in the level of adaptive mutation due to deficiency of GroE is not due to loss of viability. (A and B) Accumulation of Lac^+ revertants of *groE*$^+$ and *groEL100* mutant strains during incubation on lactose-minimal medium. Data are the cumulative number of Lac^+ colonies divided by the number of viable Lac^- cells on the plate 2 days earlier; each point is the mean ± SEM of results for 20 independent cultures (some error bars are smaller than the symbols). Because it takes 2 days for a Lac^+ revertant to make a visible colony, the values are displaced 2 days earlier to correspond to the points in the survival curves in panels C and D. (A) Circles, PFG60 (*groE*$^+$); triangles, PFG61 (*groEL100*). (B) Circles, PFG62 (Δ*recG263 groE*$^+$); triangles, PFG63 (Δ*recG263 groEL100*). All strains are also *purA*::Tn*10*. (C and D) Survival of Lac^- cells during incubation on lactose-minimal medium. Each point is the mean ± SEM of results for three independent cultures. (C) Circles, PFG60 (*groE*$^+$); triangles, PFG61 (*groEL100*). Numbers have been normalized to the value for PFG60 on day 0. (D) Circles, PFG62 (Δ*recG263 groE*$^+$); triangles, PFG63 (Δ*recG263 groEL100*). Because about 100-fold-more PFG63 cells than PFG62 cells were plated, the numbers of PFG62 cells were multiplied by 100; then the results for both strains were normalized to this value for PFG62 on day 0.

Deficiency of GroE reduces the cellular amount of DNA Pol IV. GroE is required for normal levels of SOS-dependent mutagenesis after DNA damage because the polymerase subunit of DNA Pol V, the product of the *umuC* gene, is destabilized in *groE* mutants (9). The *dinB* gene, which encodes Pol IV, is homologous to the *umuC* gene (45). Therefore, we checked whether the amount of Pol IV was also reduced in *groE* mutant cells. Because Pol IV is induced late in stationary phase (34), we grew cells in minimal medium to saturation and then incubated the cultures for an additional 16 to 24 h before preparing samples for Western blots. As shown in Fig. 3, a *groES30* mutant strain had about 1/10 the amount of Pol IV as a *groE*$^+$ strain. Pol IV levels are elevated in *recG* mutant cells (34) (Fig. 3), and the *groES30* mutant allele reduced the amount of Pol IV in these cells about threefold. Similar results were obtained with the *groEL100* and the *groES*::Cm mutant strains (data not shown). (The faster-running band in Fig. 3 is probably a degradation product; see reference 34).

GroE may affect the levels of Pol IV indirectly. We were unable to detect a direct interaction between GroE and Pol IV using coimmunoprecipitation with anti-GroEL antibody, a technique that has previously been used to detect an interaction between GroE and Pol V (10). We also failed to detect GroE copurifying on a metal resin with His-tagged Pol IV from a crude cell lysate. We used Pol IV and the peptide-binding domain of GroEL (14) in the yeast two-hybrid analysis and again failed to detect an interaction, although this technique has been used to reveal other substrates of GroE (12). In addition, the yeast two-hybrid system detects an interaction between DnaN and Pol IV (35), which we confirmed and used as a positive control in our experiments (data not shown).

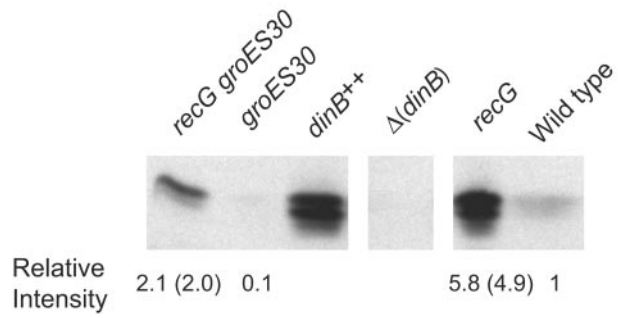

FIG. 3. A mutant allele of *groE* reduces the cellular amount of Pol IV protein. Shown is a Western blot indicating the amounts of Pol IV in (from left to right) PFG67 (*recG groES30*), PFG65 (*recG*$^+$ *groES30*), FC1230 (*dinB*$^{++}$), FC1240 (Δ*dinB*), FC526 (*recG*), and FC40 (wild type). Only one blot is shown, but lanes with irrelevant samples have been removed; the rightmost three lanes have been previously published (34). Samples consisting of 40 μg of total protein were loaded in each lane. The intensities of the bands for each strain relative to those of the wild-type strain are given below each lane; the intensity of only the upper band relative to that of the wild-type strain is in parentheses.

GroE affects the amount of Pol IV independently of LexA and when Pol IV is expressed only from the chromosome. Pol IV is induced after DNA damage and in LexA-deficient [LexA(Def)] strains in which all SOS genes are derepressed (8, 28, 34). As shown in Fig. 4, the *groEL100* allele reduced the amount Pol IV in a LexA(Def) strain about sixfold, demonstrating that GroE affects the levels of Pol IV independently of LexA.

There are two copies of *dinB* in most of our strains, one on the chromosome and one on the episome. However, the three experimental strains shown in Fig. 4 are all F$^-$, so *dinB* is expressed only from their chromosomes. Thus, the effect of GroE on Pol IV is not a peculiarity of the episome or the episomal allele but is also exerted when Pol IV is expressed solely from the chromosome.

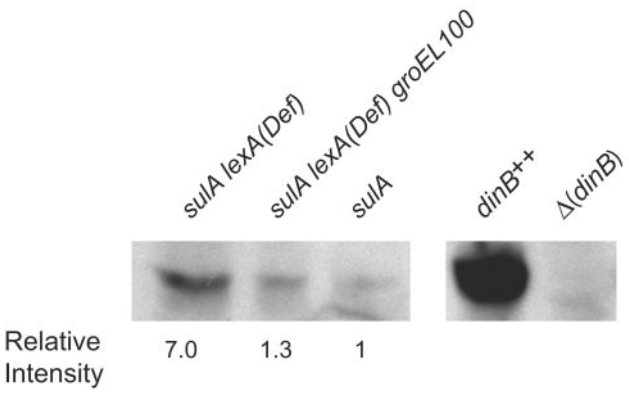

FIG. 4. A mutant allele of *groE* reduces the cellular amount of Pol IV protein in the absence of LexA. Shown is a Western blot indicating the levels of Pol IV in (from left to right): PFG324 [*sulA lexA*(Def)], PFG325 [*sulA lexA*(Def) *groEL100*], PFG326 (*sulA*), FC526 (*dinB*$^{++}$), and FC1240 [Δ(*dinB*)]. PFG324, PFG325, and PFG326 are F$^-$ strains, so their only *dinB* alleles are chromosomal; these strains are also *sulA11* [which prevents lethal filamentation due to the *lexA*(Def) allele]. Only one blot is shown, but lanes with irrelevant samples have been removed. Samples consisting of 40 μg of total protein were loaded. The intensities of the bands for each strain relative to those of the wild-type strain are given below each lane.

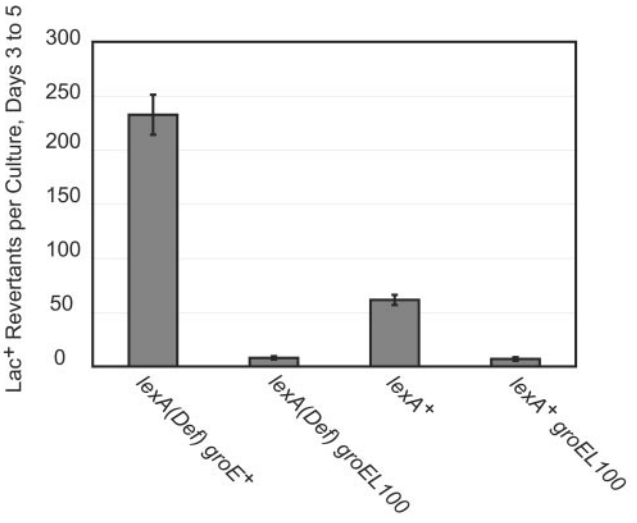

FIG. 5. A *groE* mutant allele reduces adaptive mutation in the absence of LexA. The accumulation of Lac$^+$ revertants during incubation on lactose-minimal medium is shown. Three independent cultures were plated for each strain; data are the mean numbers of Lac$^+$ colonies appearing each day from days 3 to 5 ± SEM. *lexA*(Def) *groE*$^+$ = PFG328: *lexA*(Def) *groEL100* = PFG329; *lexA*$^+$ = PFG340; *lexA*$^+$ *groEL100* = PFG341. All strains are *sulA11* and *purA*::Tn*10*.

GroE affects adaptive mutation independently of LexA. Although *dinB* is derepressed in LexA(Def) mutant strains (34), these strains normally have adaptive mutation rates lower than those of LexA$^+$ strains because another gene, *psiB*, whose product interferes with the expression of the SOS response, is also derepressed in LexA(Def) cells (41). However, in the *purA*::Tn*10* background, the LexA(Def) mutant strain showed a fourfold increase in adaptive mutation relative to that of the LexA$^+$ control (Fig. 5). Apparently, the *psiB*$^+$ phenotype is suppressed by the *purA*::Tn*10* allele or by a mutation that maps close to it. This fortuitous result allowed observation of the 30-fold reduction in the level of adaptive mutation of the LexA(Def) strain due to the *groEL100* allele (Fig. 5).

Mutations in *groE* and *dinB* are epistatic in *recG*$^+$ cells but not in *recG* mutant cells. We wished to determine whether the reduced amount of Pol IV in *groE* mutant strains was sufficient to explain their reduction in adaptive mutation. Epistasis is the classical genetic test to determine if two genes are in the same or different pathways. As shown in Fig. 6A, in *recG*$^+$ cells the *groEL100* Δ*dinB::Zeo* double mutant strain had the same low rate of adaptive mutation to Lac$^+$ as did strains carrying either single mutant. However, as shown in Fig. 6B, in *recG* mutant cells the double mutant had a significantly lower rate of adaptive mutation than either single mutant. These results support the hypotheses that, in otherwise wild-type cells, GroE affects adaptive mutation solely by regulating the amount of Pol IV, but in *recG* mutant cells, GroE has an additional effect.

GroE is unlikely to act via RpoS but does influence SOS induction. In our previous paper on the regulation of Pol IV, we reported that the general stress response sigma factor, RpoS (σ^{38}), is required for the induction and maintenance of Pol IV in stationary-phase cells. So, an obvious hypothesis is

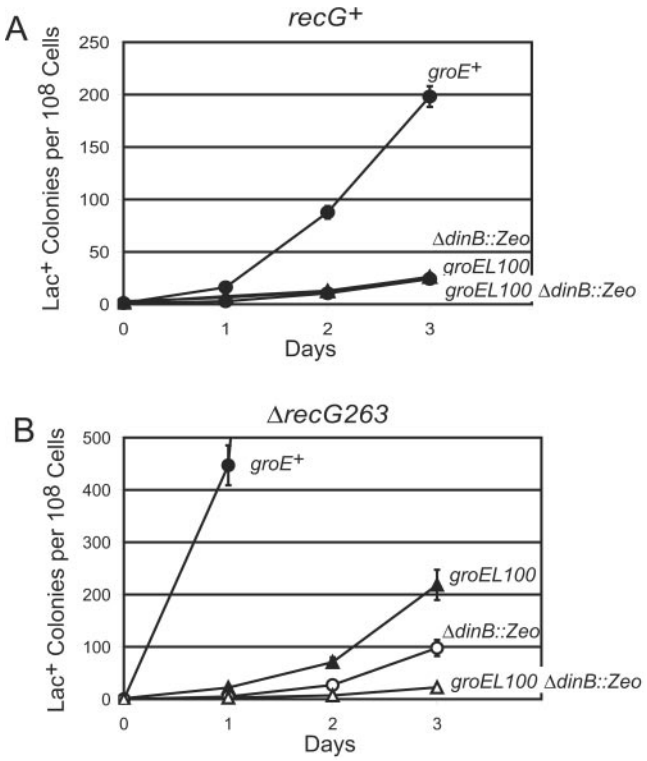

FIG. 6. The *groEL100* and Δ*dinB::*Zeo mutant alleles are epistatic in *recG*$^+$ but not in *recG* mutant strains. Shown is the accumulation of Lac$^+$ revertants of *groE*$^+$, Δ*dinB::*Zeo, *groEL100*, and *groEL100* Δ*dinB::*Zeo strains during incubation on lactose-minimal medium. Data are the cumulative number of Lac$^+$ colonies divided by the number of viable Lac$^-$ cells on the plate 2 days earlier. Because it takes 2 days for a Lac$^+$ revertant to make a visible colony, the values shown are displaced 2 days earlier. Each point is the mean ± 95% confidence limits of results for six cultures (some error bars are smaller than the symbols). (A) Closed circles, PFG60 (*groE*$^+$); open circles, PFG250 (Δ*dinB::*Zeo); closed triangles, PFG61 (*groEL100*); open triangles, PFG251 (Δ*dinB::*Zeo *groEL100*). (B) Closed circles, PFG62 (*groE*$^+$); open circles, PFG305 (Δ*dinB::*Zeo); closed triangles, PFG63 (*groEL100*); open triangles, PFG307 (Δ*dinB::*Zeo *groEL100*). For clarity, the results for PF62 after day 1 are not shown; see Fig. 2 for comparable data for this strain. All strains are also *purA*::Tn*10*.

TABLE 2. Expression of *lacZ* under the control of various promoters in different genetic backgrounds

Strain	Fusion	Relevant genotype	No. of cultures	Mean β-galactosidase activity (Miller units) ± 95% CL[a]
PFG340	*rpoS-lacZ*[b]	*groE*$^+$	3	2,053 ± 191
PFG341	*rpoS-lacZ*[b]	*groEL100*	3	2,286 ± 44
PFG342	P*rpoS-lacZ*[c]	*groE*$^+$	3	7,426 ± 873
PFG343	P*rpoS-lacZ*[c]	*groEL100*	3	8,031 ± 1,186
PFG334	*katE-lacZ*[d]	*groE*$^+$	7	662 ± 76
PFG335	*katE-lacZ*[d]	*groEL100*	7	461 ± 99
PFG336	*katE-lacZ*[d]	*groE*$^+$ *rpoS*::Cm	3	23 ± 3
PF2253	P*groE-lacZ*[e]	*rpoS*$^+$	7	585 ± 164
PF2259	P*groE-lacZ*[e]	*rpoS*::Cm	7	361 ± 41
PF2252	P_L-*lacZ*[f]	*rpoS*$^+$	7	6,112 ± 261
PF2258	P_L-*lacZ*[f]	*rpoS*::Cm	7	5,525 ± 1,194
PFG308	*sulA-lacZ*[g]	*groE*$^+$	7	224 ± 59
PFG309	*sulA-lacZ*[g]	*groEL100*	7	168 ± 28
PFG332	*sulA-lacZ*[g]	*recG groE*$^+$	6	610 ± 339
PFG333	*sulA-lacZ*[g]	*recG groEL100*	7	256 ± 38

[a] Values are the means of results with the indicated number of independent cultures ± the 95% confidence limits (CL). Results from several experiments have been combined.

[b] *rpoS-lacZ* is a gene fusion of *lacZ* to *rpoS* under the control of the *rpoS* promoter (26).

[c] P*rpoS-lacZ* is an operon fusion that puts *lacZ* under the control of the *rpoS* promoter (26).

[d] *katE-lacZ* is λ(*katE::lac* Kn) that puts *lacZ* under the control of the σ^{38}-dependent *katE* promoter (53).

[e] P*groE-lacZ* is an operon fusion that puts *lacZ* under the control of the σ^{32}-dependent *groE* promoter; the fusion does not retain the σ^{70}-dependent *groE* promoter (61).

[f] P_L-*lacZ* is an operon fusion that puts *lacZ* under the control of the σ^{70}-dependent lambda promoter (61).

[g] *sulA-lacZ* is an operon fusion of *Mu*d(Ap *lac* B::Tn*9*) to the *sulA* gene (D. Mount, personal communication).

that GroE and RpoS act together in a single pathway affecting Pol IV. However, we were unable to construct stable *rpoS groES* or *rpoS groEL* double mutants to test this hypothesis directly by using epistasis. Therefore, we used ectopic *lacZ* fusions to determine if mutations in each gene influence the expression of the other gene. For these experiments we grew the cells under the same conditions that were used to determine the amount of Pol IV protein.

Since Pol IV levels are more severely reduced by a GroE defect than by loss of RpoS (34), it is unlikely that our results can be explained by a positive effect of GroE on RpoS. In confirmation, the *groEL100* allele did not decrease, and in fact slightly increased, the expression of *lacZ* transcriptional and translational fusions to *rpoS* (Table 2). However, when RpoS activity was measured by using a *lac*Z fusion to *katE,* a gene whose expression is *rpoS* dependent (53), the *groEL100* allele resulted in a 30% decrease in β-galactosidase activity (Table 2). Although statistically significant ($t = 3.9$; $P = 0.002$), this reduction is far less than the 97% decrease in *katE* expression caused by total loss of RpoS (Table 2) and seems of insufficient magnitude to account for the 10-fold reduction in the levels of Pol IV observed in *groE* mutant strains (Fig. 3).

Our Pol IV results also could be explained if RpoS were a positive effector of GroE, although there are no reports of this in the literature. As shown in Table 2, the *rpoS::*Cm allele caused about a 40% reduction in the expression of a *lacZ* fusion to the *groE* heat shock (σ^{32}-dependent) promoter (Table 2), and this reduction is statistically significant ($t = 3.2$; $P = 0.01$). Again, the magnitude of this effect seems insufficient to account for the 10-fold reduction in the levels of Pol IV caused by GroE deficiency (Fig. 3).

Using a *lacZ* fusion to the SOS-induced gene, *sulA*, we also tested whether GroE was required for expression of the SOS response. As shown in Table 2, in wild-type cells the *groEL100* allele reduced *sulA* expression by about 25%, but this reduction was significant, with a probability of only 8% ($t = 1.9$; $P = 0.08$). Interestingly, in *recG* mutant cells in which the SOS response is partially induced (38) (Table 2), GroE deficiency resulted in a larger decrease (58%), and this decrease was statistically significant despite the variability of results with the *sulA-lacZ* fusion ($t = 3.16$; $P = 0.03$). Thus, the 20-fold reduction in the level of adaptive mutation of *recG* mutant strains caused by a deficiency in GroE (Fig. 2) can be attributed to both a reduction in the amount of Pol IV and a decrease in the degree of SOS induction.

DISCUSSION

The heat shock response in *E. coli* is induced by temperature shift and other conditions that result in unfolded proteins. Approximately 30 genes are positively regulated by the heat shock sigma factor RpoH (σ^{32}). GroE, *E. coli*'s Hps60 chaperone, is part of this regulon and is required at all temperatures to aid essential proteins to fold and maintain their proper conformation (40). GroE consists of two subunits that in *E. coli* are encoded by the *groES/groEL* operon; both genes are essential, and mutation of each results in the same phenotypes (13, 58, 66). In addition to its RpoH-dependent promoter, the *groES/groEL* operon has a promoter recognized by the vegetative sigma factor, RpoD (σ^{70}), allowing for expression of GroE at all temperatures (67). GroE and certain other components of the heat shock regulon are also induced by DNA damage, oxidative stress, antibiotics and heavy metals, phage infection, and carbon source or amino acid starvation (reviewed in reference 64). Thus, the RpoH regulon can be considered a general stress response. The discovery, reported here, that cellular levels of DNA Pol IV are dependent on GroE provides a new link between this error-prone polymerase, adaptive mutation, and global stress responses.

GroE is required for the recovery of mutations after DNA-damaging treatments that induce the SOS response (9, 37). There is strong evidence that GroE interacts with the polymerase subunit of Pol V and protects it from degradation (10). Because Pol IV is a homolog of Pol V, it seemed likely that GroE would also interact with Pol IV, but we failed to detect an interaction between GroE and Pol IV using three methods: coimmunoprecipitation, copurification on a metal resin, and yeast two-hybrid analysis. Possibly, our techniques were not robust enough to detect an interaction; alternatively, the effect of GroE on Pol IV levels may be indirect. For example, some substrate of GroE may be required for Pol IV stability.

GroE appears to affect expression of the SOS response, particularly in *recG* mutant cells (Table 2). Mass coimmunoprecipitation experiments identified RecA as a GroE substrate (27). Since RecA promotes the proteolytic inactivation of LexA (36), it is possible that when GroE is deficient, levels of RecA are low, levels of active LexA are relatively high, and SOS genes, including *dinB*, are repressed. However, this sequence of events cannot account for the entire effect of GroE because even in the absence of LexA, a deficiency in GroE reduced both the cellular amount of Pol IV and the rate of adaptive mutation (Fig. 4 and 5).

It is unlikely that GroE affects Pol IV via RpoS for the following reasons. First, we could find no reports in the literature that GroE is a positive effector of the amount or activity of RpoS. Second, a defect in GroE reduces the amount of Pol IV to a greater extent than does the loss of RpoS (34); if GroE were a positive effector of RpoS, the loss of RpoS should have the greater impact. Third, the *groEL100* mutation had only a modest effect on the expression and activity of RpoS (Table 1). On the other hand, the results in Table 2 suggest that RpoS may increase the transcription of the *groE* operon from its heat shock promoter by about 40%, suggesting that RpoS may be a modest positive effector of GroE. However, these small effects may not be biologically significant and are unlikely to account for the large effects that GroE has on levels of Pol IV.

Although it appears that RpoS and GroE do not positively affect each other, it is still possible that they are in the same pathway that regulates Pol IV. For example, each may be a positive effector of yet a third component that is itself a positive effector of Pol IV. Further experiments are necessary to test this hypothesis.

Deficiency in GroE had a greater relative effect on Pol IV in *recG* mutant strains than in wild-type strains (Fig. 1 and 2). The results of epistasis tests (Fig. 6) indicate that in *recG*$^+$ cells, the reduction in Lac$^+$ adaptive mutations when GroE is deficient is due solely to reduction in the amount of Pol IV, but this is not the case in *recG* mutant cells. Interestingly, the adaptive mutation rate of the *recG groEL100* mutant strain was nearly the same as that of the *recG*$^+$ *groE*$^+$ strain (for example, see Fig. 6), yet from the Western results shown in Fig. 3, the double mutant has twice the amount of Pol IV. Thus, the large decrease in adaptive mutation caused by deficiency of GroE in *recG* mutant strains can be attributed to a reduced amount of Pol IV (Fig. 3) plus a decrease in the degree of SOS induction (Table 1). The LexA-repressed genes that are known to be required for adaptive mutation are *recA* and *ruvAB* (6, 17, 20, 23, 24); in addition there may be yet-unknown SOS genes involved. To determine how GroE affects expression of SOS-induced genes requires further experimentation.

Overproduction of Pol IV is a powerful mutator (29, 30, 60), but deletion of Pol IV has little effect on growth-dependent spontaneous mutation rates (42, 51, 62; P. L. Foster, unpublished results), although it does contribute to spontaneous mutation on extrachromosomal elements, such as the episome (32; Foster, unpublished). These observations suggest that Pol IV levels and activity are normally tightly regulated and targeted. Previously we reported that Pol IV is induced late in stationary phase and that RpoS, the general stress response sigma factor, is required for this induction and for the continued maintenance of high levels of Pol IV during starvation (34). The results presented here show that levels of Pol IV are sustained, directly or indirectly, by GroE. Since GroE is part of the RpoH regulon, which is induced by a variety of stresses that perturb or prevent growth, our results suggest that it is important to survival to have adequate levels of this error-prone polymerase. Indeed, all three of *E. coli*'s inducible DNA polymerases, Pol II, IV, and V, confer a competitive advantage during prolonged stationary phase (63). The links between Pol IV and global responses to DNA damage, protein damage, and starvation provide evidence that the ability to increase genetic diversity has evolved to promote survival during stress.

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